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Mermithid parasitism in Ceratopogonidae: A literature review and critical assessment of host impact and potential for biological control of *Culicoides*

Мермитиды как паразиты мокрецов (Ceratopogonidae): обзор литературы и критическая оценка воздействия мермитид на хозяев, а также возможностей использования мермитид для биологического контроля представителей рода *Culicoides*

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КЛЮЧЕВЫЕ СЛОВА: Mermithidae, *Heleidomermis*, Ceratopogonidae, мермитиды, мокрецы, *Culicoides*, паразитирование, биологический контроль.

ABSTRACT. The worldwide literature on mermithid associations with adults and larvae of Ceratopogonidae is reviewed, including a number of studies from the former USSR and one important Chinese study hitherto unknown to most researchers. Although frequently misidentified or not identified beyond family, mermithids probably are parasites in biting midges worldwide, and more species exist in a wider variety of habitats than are now understood. *Aproctonema* (the closely related family Tetradonematidae) and seven existing genera of Mermithidae have been reported from Ceratopogonidae: *Agamomermis*, *Ceratomermis*, *Gastromermis*, *Heleidomermis*, *Limnomermis*, *Romanomermis*, and *Spiculimermis*. Reports are provided from 40 *Culicoides* spp. in 11 subgenera. Most are probably *Heleidomermis* spp., which utilize multiple *Culicoides* spp. The general life histories of the three valid *Heleidomermis* spp. (*H. cataloniensis*, *H. magnapapula* and *H. vivipara*) are described and compared. The stereotypical habitat is silty, surface mud at the edges of shallow somewhat polluted or stagnant water. They are generally larval parasites, infecting early stage hosts and emerging from the last (4th) instar, but carryover into the adult stage can be common and probably important for dispersal. Field parasitism of up to 67–69% of larvae is documented for all three *Heleidomermis* spp. Data from an experimental field release trial in California, USA are presented, which caused an 84% reduction in emergence of *Culicoides sonorensis* due to *H. magnapapula*. Advantages and disadvantages of *Heleidomermis* spp. for biological control of *Culicoides* are listed. Introduction of mermithids reared *in vivo* or via transfer from known field sites could assist the inherently limited dispersal of these key parasites.

РЕЗЮМЕ. Выполнен обзор литературы о связях мермитид (Nematoda: Mermithida) с личинками и имаго мокрецов (Diptera: Ceratopogonidae). Обзор охватывает всю мировую литературу по данному вопросу, включая ряд работ из бывшего СССР и важную работу из Китая, которые были неизвестны большинству исследователей. По-видимому, мермитиды встречаются в мокрецах во всех регионах мира, а спектр заселяемых ими биотопов и число видов, паразитирующих в мокрецах, существенно шире того, что известно к настоящему времени. Литературные данные по мермитидам — паразитам мокрецов содержат значительное число неточных или ошибочных определений. В качестве паразитов Ceratopogonidae отмечены представители 7 современных родов семейства Mermithidae (*Agamomermis*, *Ceratomermis*, *Gastro-*

mermis, *Heleidomermis*, *Limnomermis*, *Romanomermis* и *Spiculimermis*) и род *Aproctonema* из близкого семейства Tetradonematidae. Имеются указания о паразитировании мермитид в 40 видах рода *Culicoides*, относящихся к 11 подродам. Большая часть этих находок, по-видимому, относится к видам рода *Heleidomermis*, которые паразитируют на многих видах *Culicoides*. В сравнительном аспекте анализируется биология и жизненный цикл трёх валидных видов рода *Heleidomermis* — *H. cataloniensis*, *H. magnapapula* и *H. vivipara*. Типичный биотоп этих видов — верхний слой ила по краям мелких стоячих или несколько загрязненных водоёмов. Виды *Heleidomermis* — паразиты личинок мокрецов, обычно заражающие личинок младших возрастов и покидающие личинку последнего (4-го) возраста. При этом нередко наблюдается переход паразита во взрослых мокрецов; по-видимому, он важен для расселения мермитид. Экстенсивность заражения личинок хозяев в природе, достигающая 67–69%, отмечена для всех трёх видов *Heleidomermis*. Представлены данные полевого эксперимента, проведённого в Калифорнии, в котором было продемонстрировано снижение числа вылетающих имаго *Culicoides sonorensis* на 84% при заражении их личинок *H. magnapapula*. Анализируются преимущества и недостатки использования *Heleidomermis* spp. для биологического контроля *Culicoides*. Интродукция мермитид, полученных *in vivo* или из естественных местообитаний, может быть использована для расселения этих паразитов, в ряде случаев играющих ключевую роль для контроля популяций кровососущих мокрецов.

Introduction

Forty years ago, as part of her pioneering studies into the morphology and ecology of immatures of biting midges, Dr. Valentina Glukhova reported parasitism in both adults and larvae of *Culicoides* spp. in the Soviet Union by nematodes in the superfamily Mermithoidea [Glukhova, 1967]. It was a very short paper — only 2 pages long. That paper was nevertheless very important for several reasons. Prior to that time, as discussed below and listed in Table 1, the literature contained a number of scattered observations mostly on parasitism of adult *Culicoides* by mermithid nematodes, and that parasitism sometimes resulted in formation of the entomological oddities known as intersexes. V.M. Glukhova made the scientific community explicitly aware that this mermithid parasitism originated in the larval stage of the host. Further, levels of parasitism were sometimes rather high (up to 27%), and multiple *Culicoides* species in the same habitat could harbour the nematodes, which often emerged from the host larvae and killed them in the process. The range and type of habitats harbouring the immature *Culicoides* and the nematodes were described for the first time as well. This discovery by Dr. Glukhova led to the description of a new genus of mermithids, *Heleidomermis*. Three valid species of *Heleidomermis* now are known, and two of them (*H. vivipara* Rubzov and *H. magnapapula* Poinar & Mullens) are well documented as key natural enemies of potential use as biological control agents for this important group of midges (see below). It therefore is quite appropriate that we dedicate this review and discussion of mermithids in ceratopogonids to the memory of Dr. Valentina Glukhova, a dedicated and capable scientist who first exposed critical aspects of their unique life cycles to science.

Mermithids in adult Ceratopogonidae

Most of the earliest reports of mermithids were from adult midges, and we now know that mermithids enter their hosts when the midges are in their larval stage. Due to factors discussed later, such as parasitism of an older stage larva, in some cases the parasites persist through the pupal stage and into the adult midge. It is not known whether the mermithid continues to develop in the ceratopogonid pupa, feeding on the pupal tissues and body fluids while the new adult midge tissues are formed, or whether the nematodes may spend this period in stasis. Once in the adult the mermithid is generally coiled in the adult midge abdomen (Figs 1A–D) and reaches maturity there.

When and where emergence from the adult takes place also is not known, but presumably the parasitized midge would fly near or alight on habitats adequate for the nematode, which then might sense a suitable habitat (for instance, water or high humidity levels) and quickly emerge. Such behaviour has been described, for example, in Simuliidae, where mermithid-infested castrated adults (both females and males) fly to oviposition sites and deposit mermithids among the eggs laid by other simuliid adults [Rubzov, 1974a]. While most mermithids seem to favour infecting and emerging from the larval host stage of ceratopogonids, carryover into the adult stage can be common and is likely to be more frequent than generally presumed. In fact, this carryover into adults probably serves as an important dispersal vehicle for some or perhaps even all known parasitic mermithids from ceratopogonids.

The first time mermithids were cited as commonly parasitizing adult ceratopogonid midges goes back to 1938, when Buckley in Malaysia found mermithid nematodes in the abdomens of six *Culicoides* species (Table 1). An older observation by Kieffer [1914] of intersexes in males of *Forcipomyia allocera* was mistakenly attributed to mermithid parasitism by Thienemann [1954] (see below). Since the pioneering studies of Keilin [1921] (in *Dasyhelea* larvae) and Buckley [1938] (in *Culicoides* adults), additional reports of mermithid parasitism (for both midge larvae and/or adults) have been contributed by many authors (see summary list in Table 1). Worth mentioning are the previous reviews by Poinar [1975] and Wirth [1977a, 1977b]; Strand & Wirth [1977] added comments to the same literature list given by Wirth [1977b].

Table 1. Reports[1] of parasitism of Ceratopogonidae by members of the nematode Order Mermithida[2].
Таблица 1. Данные о паразитировании нематод из отряда Mermithida в мокрецах (Ceratopogonidae).

Region and Locality / Habitat of Host Immatures	Host(s) Parasitized	Parasite / Prevalence[3]	References
UK (England) / decomposed sap filling the wound of an elm tree	Larva of *Dasyhelea flavifrons* (Guérin, 1833) (quoted as *D. obscura* (Winnertz, 1852))	maybe Mermithidae / few	Keilin, 1921
Malaysia (Kuala Lumpur)	Adult of *Culicoides jacobsoni* Macfie, 1934 (quoted as *C. buckleyi* Macfie, 1937), *C. obscurus* Tokunaga & Murachi, 1959 (quoted as *C. pungens* de Meijere, 1909), *C. orientalis* Macfie, 1932, *C. oxystoma* Kieffer, 1910, *C. peregrinus* Kieffer, 1910, and *C. shortti* Smith & Swaminath, 1932	Mermithidae / NL	Buckley, 1938
India	Adult of *Culicoides oxystoma* Kieffer, 1910 (quoted as *C. alatus* Das Gupta & Ghosh, 1956)	*Mermis* Dujardin, 1842 / NL (probably Mermithidae)	Sen & Das Gupta, 1958
USA (NW Florida)	Several adult *Culicoides crepuscularis* Malloch, 1915, one adult *C. haematopotus* Malloch, 1915	Mermithidae / NL	Beck, 1958
France (Strasbourg)	Adult of *Culicoides kibunensis* Tokunaga, 1937 (misidentified as *C. albicans* (Winnertz, 1852); later corrected by Callot & Kremer [1963] and quoted by them as *C. cubitalis* Edwards, 1939)	Mermithidae / approx. 5% (indistinctively referred to as *Agamomermis* Stiles, 1903 or *Mermis* Dujardin, 1842)	Callot, 1959
USSR (Transcaucasia)	Larva of *Culicoides* Latreille, 1809	Mermithidae / NL	Dzhafarov, 1962, 1964
France (Strasbourg)	Adults of *Culicoides fascipennis* (Staeger, 1839), *C. festivipennis* Kieffer, 1914 (quoted as *C. odibilis* Austen, 1921), *C. kibunensis* Tokunaga, 1937 (quoted as *C. cubitalis* Edwards, 1919), and *C. obsoletus* (Meigen, 1818)	Mermithidae (indistinctively referred to as *Agamomermis* Stiles, 1903 or *Mermis* Dujardin, 1842) / 8% (*C. fascipennis*) - 16% (*C. obsoletus*)	Callot & Kremer, 1963
France (Charente-Maritime)	Adult of *Culicoides alazanicus* Dzhafarov, 1961 (quoted as *C. musilator* Kremer & Callot, 1961)	Most likely Mermithidae / 5%	Callot & Kremer, 1963
France (Strasbourg area?) / tree holes	Larva of *Dasyhelea* Kieffer, 1911	Mermithidae / NL	Callot & Kremer, 1963
India (Calcutta)	A single adult of *Atrichopogon* Kieffer, 1906	*Mermis* Dujardin, 1842 / NL (probably Mermithidae)	Das Gupta, 1964
Mexico (Carmen Island, Baja California)	One adult female of *Leptoconops kerteszi* Kieffer, 1908	Mermithidae / NL	Whitsel, 1965
USA (Florida: Gainesville)	One adult male of *Culicoides stellifer* (Coquillett, 1901)	Mermithidae / NL	Smith, 1966
USA (Florida: Alachua County)	Adult of *Culicoides crepuscularis* Malloch, 1915, *C. haematopotus* Malloch, 1915, and *C. stellifer* (Coquillett, 1901)	Mermithidae / to 30-90%	Smith & Perry, 1967

Table 1. Continued.
Таблица 1. Продолжение.

Region and Locality / Habitat of Host Immatures	Host(s) Parasitized	Parasite / Prevalence[3]	References
USSR (SE Kazakhstan: Alma-Ata Prov., Ili River Valley)	Adult of *Culicoides pulicaris* (Linnaeus, 1758) (could also be *C. deltus* Edwards, 1939 or especially *C. punctatus* (Meigen, 1804) as discussed by Glukhova, 1989)	*Ceratomermis heleis* (Rubzov, 1967) / NL (as *Agamomermis* Stiles, 1903)	Glukhova, 1967; Rubzov, 1967, 1972, 1978
USSR (Azerbaijan)	Adult and larva of *Leptoconops* Skuse, 1889	Mermithoidea / NL	Glukhova, 1967
USSR (Ukraine: Lvov and Kharkov provinces; NW Russia: Pskov Prov.; SE Kazakhstan: Alma-Ata Prov.)	Larvae of several species of *Culicoides* Latreille, 1809	Mermithoidea / NL	Glukhova, 1967
USSR (NW Russia: Karelia) / muddy pools near livestock watering areas	Larva of *Culicoides nubeculosus* (Meigen, 1830), *C. puncticollis* (Becker, 1903) and *C. stigma* (Meigen, 1818)	Mermithoidea / NL	Glukhova, 1967
USSR (NW Russia: southern Karelia) / marshy river bank	Larva of *Culicoides circumscriptus*-group	Mermithoidea / NL	Glukhova, 1967
USSR (NW Russia: southern Karelia) / boggy path in pine forest with *Sphagnum*	Larva of *Culicoides grisescens* Edwards, 1939	Mermithoidea (oviparous) / 18-27%	Glukhova, 1967
USA (SW Louisiana) / tree holes	Larva of *Culicoides nanus* Root & Hoffman, 1937	Mermithidae / 1-10%	Chapman et al., 1968
USA (SW Louisiana) / tree holes	Larva of *Culicoides arboricola* Root & Hoffman, 1937	*Aproctonema chapmani* Nickle, 1969[4] / NL	Nickle, 1969; Chapman et al., 1969; Chapman, 1973
USA (SW Louisiana) / tree holes	Larva of *Culicoides nanus* Root & Hoffman, 1937	*Romanomermis* Coman, 1961 / NL (probably erroneous genus-level identification)	Chapman et al., 1969; Chapman, 1973
UK (England: Surrey)	Adult of *Culicoides obsoletus* (Meigen, 1818)	Mermithidae / NL, but common	Boorman & Goddard, 1970
USSR (NW Russia: southern Karelia) / muddy organic-rich pool in livestock watering area	Larva of *Culicoides nubeculosus* (Meigen, 1830) and *C. stigma* (Meigen, 1818)	*Heleidomermis vivipara* Rubzov, 1970 / NL	Rubzov, 1970, 1972, 1978 (based on the material mentioned by Glukhova, 1967)
USSR (Russia, Buryatia: Tunkin Valley) / marshy region next to forest stream	Larva of *Culicoides helveticus* Callot, Kremer & Deduit, 1962	*Heleidomermis ovipara* Rubzov, 1974[5] / to 67% (as "Mermithoidea")	Mirzaeva, 1971
USSR (Russia, Buryatia: valleys of rivers Tunkin and Irkut) / river side channels, marshy pools, marshy river banks, wet lake shores with moss	Larva of *Culicoides fascipennis* (Staeger, 1839), *C. festivipennis* Kieffer, 1914 (quoted as *C. odibilis* Austen, 1921), *C. grisescens* Edwards, 1939, *C. manchuriensis* Tokunaga, 1941, *C. pulicaris* (Linnaeus, 1758), *C. sibiricus* Mirzaeva, 1964 and *C. sphagnumensis* Williams, 1955	Mermithoidea / NL	Mirzaeva, 1971
UK (England)	Three *obsoletus*-group adult *Culicoides* and two adult *C. pictipennis* (Staeger, 1839)	Mermithidae / NL	Service, 1974
USSR (Russia, Buryatia: Tunkin Valley) / habitats were listed in Mirzaeva, 1971 (see above)	Larva of (mostly) *Culicoides helveticus* Callot, Kremer & Deduit, 1962; also larva of *C. circumscriptus* Kieffer, 1918, *C. manchuriensis* Tokunaga, 1941, *C. pulicaris* (Linnaeus, 1758) and *Culicoides* sp.; and (rarely) pupa of *Culicoides*	*Heleidomermis ovipara* Rubzov, 1974[5] / NL	Rubzov, 1974b, 1978

Table 1. Continued.
Таблица 1. Продолжение.

Region and Locality / Habitat of Host Immatures	Host(s) Parasitized	Parasite / Prevalence[3]	References
USSR (NW Russia: Pskov Prov.) / lake (probably, near shore)	Larva of *Bezzia* Kieffer, 1899	*Gastromermis bezzii* Rubzov, 1974 (female)	Rubzov, 1974b
USSR (NW Russia: Pskov Prov.) / river	Unidentified ceratopogonid larva (probably Palpomyiinae)	*Agamomermis gluchovae* Rubzov, 1974 (female)	Rubzov, 1974b
USSR (Russia, Buryatia: Tunkin Valley) / lake shore with sedges, horsetail and moss	Larva of *Culicoides* Latreille, 1809 (according to personal communication by Mirzaeva; quoted as "Ceratopogonidae")	*Spiculimermis mirzajevae* Rubzov, 1974 (female) / NL	Rubzov, 1974b (based on the material by Mirzaeva)
USSR (Russia, Buryatia) / bank of Irkut River	Larva of *Culicoides* Latreille, 1809 (according to personal communication by Mirzaeva; quoted as "Ceratopogonidae")	*Spiculimermis* Artyukhovsky, 1963 (sp. 2, undetermined) / NL	Rubzov, 1974b (based on the material by Mirzaeva)
USSR (Belorussia: Minsk)	Adult females of *Culicoides obsoletus* (Meigen, 1818)	Mermithoidea / 23%	Trukhan, 1975
Zimbabwe	Adult of *Culicoides inornatipennis* Carter, Ingram & Macfie, 1920, *C. leucostictus* Kieffer, 1911 (quoted as *C. praetermissus* Carter, Ingram & Macfie, 1920, *C. pycnostictus* Ingram & Macfie, 1925 and *C. ravus* de Meillon, 1936), and *C. tropicalis* Kieffer, 1913 (quoted as *C. babrius* de Meillon, 1943)	Mermithoidea / 0.1% (*C. ravus*) to 15.5% (*C. inornatipennis*)	Phelps & Mokry, 1976
China (Chungking)	Adult of *Culicoides riethi* Kieffer, 1914	Mermithidae / 2%	Jeu, 1977
USSR (Far Eastern Russia: Primorie Territory) / holes in broad-leaved trees	Larva of *Culicoides dendrophilus* Amossova, 1957	Mermithoidea / NL	Glukhova, 1979
USSR (NW Russia: Leningrad Prov.) / drainage from *Sphagnum* bog in wet forest	Larva of *Palpomyia* Meigen, 1818	Mermithoidea / about 100%	Glukhova, 1979
USA (New York State: Ithaca area)	Adult of *Culicoides variipennis* (Coquillett, 1901)	Mermithidae / NL (assumed to be *Heleidomermis magnapapula* Poinar & Mullens, 1987)	Mullens & Schmidtmann, 1982
USA (central and western New York State) / silty, open, manure-polluted mud from pond or stream edges near beef/dairy cattle	Larva of *Culicoides variipennis* (Coquillett, 1901)	*Heleidomermis magnapapula* Poinar & Mullens, 1987 / to 54%	Mullens & Rutz, 1982; Poinar & Mullens, 1987
USSR (Uzbekistan: Tashkent Prov.) / habitats: see under Saidalieva, 1985	Larva of *Culicoides circumscriptus* Kieffer, 1918, *C. desertorum* Gutsevich, 1959, *C. puncticollis* (Becker, 1903), and *C. salinarius* Kieffer, 1914; adult of *Culicoides* Latreille, 1809	*Heleidomermis vivipara* Rubzov, 1970 / 20-35% of larvae	Gafurov & Saidalieva, 1984
USSR (Tajikistan)	Adult of *Culicoides* (?) Latreille, 1809	*Heleidomermis vivipara* Rubzov, 1970 / NL	Gafurov & Saidalieva, 1984
USSR (Uzbekistan: Tashkent Prov., mountains) / temporary pools under faucets near human dwellings at 1000 m above sea level	Larva and adult of *Culicoides circumscriptus* Kieffer, 1918 and *C. desertorum* Gutsevich, 1959	*Heleidomermis vivipara* Rubzov, 1970 / 35% of larvae (Saidalieva, 1985), 61% of larvae (Saidalieva, 1986)[6]; 48% of adults reared from pupae (Saidalieva, 1985, 1986)	Saidalieva, 1985, 1986
USSR (Uzbekistan: Tashkent Prov., plain) / pool in unforested river flood plain	Larva of *Culicoides puncticollis* (Becker, 1903)	*Heleidomermis vivipara* Rubzov, 1970 / from solitary to 24% (Saidalieva, 1985), 35% (Saidalieva, 1986)[6]	Saidalieva, 1985, 1986

Table 1. Continued.
Таблица 1. Продолжение.

Region and Locality / Habitat of Host Immatures	Host(s) Parasitized	Parasite / Prevalence[3]	References
USSR (SE Kazakhstan: Alma-Ata and Taldykorgan provinces, Ili River Valley; Zhambyl Prov., Tasulkol' Reservoir) / brackish pools between dunes in the Ili flood plain; temporary pools in flood zone of reservoir	Late instar larvae of *Culicoides circumscriptus* Kieffer, 1918, *C. desertorum* Gutsevich, 1959, *C. festivipennis* Kieffer, 1914 (quoted as *C. odibilis* Austen, 1921), *C. puncticollis* (Becker, 1903) and *C. riethi* Kieffer, 1914	*Heleidomermis vivipara* Rubzov, 1970, *H. ovipara* Rubzov, 1974[5] / 2-5%	Gubaidulin & Bekturganov, 1986; Gubaidulin et al., 1987; Komardina & Gubaidulin, 1989
USSR (Uzbekistan)	Larva of *Culicoides puncticollis* (Becker, 1903)	*Heleidomermis vivipara* Rubzov, 1970 / 11%	Lebedeva, 1986
USSR (Uzbekistan: Tashkent, Syr-Daria and Navoi provinces) / shallow permanent, sunlit bodies of water with grassy vegetation, situated on plains, in foothills and in mountains (from slowly flowing to standing, from freshwater to saline, and from clean to polluted by runoff from cattle-farm)	Larva, pupa and adult of *Culicoides puncticollis* (Becker, 1903) (mostly); also larva (and, possibly, pupa and adult) of *C. circumscriptus* Kieffer, 1918, *C. desertorum* Gutsevich, 1959, *C. odiatus* Austen, 1921 (as *C. lailae* Khalaf, 1961), *C. punctatus* (Meigen, 1804) (as "*C. pulicaris punctata*"), and non-bloodsucking Ceratopogonidae	*Heleidomermis vivipara* Rubzov, 1970 / 1-50% (in larvae), 10-40% (in pupae), 11% (in adults)	Lebedeva, 1987a, 1987b, 1988, 1993; Lebedeva & Gafurov, 1994
USSR (Uzbekistan: Tashkent and Syr-Daria provinces) / bodies of water on plains	Larva of *Culicoides puncticollis* (Becker, 1903)	*Agamomermis* sp. 1, *A.* sp. 2 (postparasitic larvae; two undescribed new species) / solitary	Lebedeva, 1988
USA (southern California) / silty, open, manure-polluted mud from pond edges near dairy cattle	Larva and adult of *Culicoides sonorensis* Wirth & Jones, 1957	*Heleidomermis magnapapula* Poinar & Mullens, 1987 / 10% of larvae	Poinar & Mullens, 1987
USA (SW Virginia) / highly saline habitats	Larva of *Culicoides occidentalis* Wirth & Jones, 1957	*Heleidomermis magnapapula* Poinar & Mullens, 1987 / NL, but uncommon	Poinar & Mullens, 1987; J. Vaughan, personal communication
USA (Alabama) / mud near horse watering trough	Larva of *Culicoides variipennis* (Coquillett, 1901) (could be *C. sonorensis* Wirth & Jones, 1957)	*Heleidomermis* Rubzov, 1970 / 51% (probably *H. magnapapula* Poinar & Mullens, 1987)	Hribar & Murphree, 1987
USSR (Far Eastern Russia: Khabarovsk Territory) / mineralized body of water with runoff from piggery and cow barn	Larva of *Culicoides riethi* Kieffer, 1914	Mermithoidea / to 16%	Mirzaeva, 1989
USSR (SE Kazakhstan)	Larva of *Culicoides circumscriptus* Kieffer, 1918, *C. desertorum* Gutsevich, 1959, *C. festivipennis* Kieffer, 1914 (quoted as *C. odibilis* Austen, 1921), *C. pulicaris* (Linnaeus, 1758), *C. puncticollis* (Becker, 1903), and *C. riethi* Kieffer, 1914	*Heleidomermis vivipara* Rubzov, 1970 and *Ceratomermis heleis* (Rubzov, 1967) / 2-5%	Dubitskij et al., 1990 (probably, based mostly on the same data as Gubaidulin & Bekturganov, 1986, and Gubaidulin et al., 1987 – see above)
USSR (SE Kazakhstan: Alma-Ata, Taldykorgan and Zhambyl provinces) / occurred mostly in floodplain bodies of water in foothills and near large rivers in desert and semidesert zones of plains (Ili, Chu); abundant in shallow standing brackish pools with sandy shores, muddy and polluted with organic material	Larvae of the same *Culicoides* species as indicated by Gubaidulin & Bekturganov, 1986, and Gubaidulin et al., 1987	*Heleidomermis vivipara* Rubzov, 1970 / 2-30%	Bekturganov, 1991

Table 1. Continued.
Таблица 1. Продолжение.

Region and Locality / Habitat of Host Immatures	Host(s) Parasitized	Parasite / Prevalence[3]	References
Kazakhstan (SE part: Taldykorgan and Alma-Ata provinces) / pools in the Ili River flood plain	Larva of *Culicoides festivipennis* Kieffer, 1914 (quoted as *C. odibilis* Austen, 1921)	*Ceratomermis heleis* (Rubzov, 1967) (in the latter paper, as *Agamomermis* Stiles, 1903) / solitary	Bekturganov, 1991; Gubaidulin & Bekturganov, 2001
USA (southern to northern California)	Larva and adult of *Culicoides sonorensis* Wirth & Jones, 1957 (as *C. variipennis* (Coquillett, 1901))	*Heleidomermis magnapapula* Poinar & Mullens, 1987 / to 69% of larvae; <<1% of adults	Paine & Mullens, 1994
USA (Georgia)	Adult of *Culicoides stellifer* (Coquillett, 1901)	Mermithidae / 0.05% (probably *Heleidomermis magnapapula* Poinar & Mullens, 1987)	McKeever et al., 1997; Brickle et al., 2008
Kazakhstan (SE part: Taldykorgan, Zhambyl and Alma-Ata [=Almaty] provinces) / lakes in Alma-Ata Prov.; other habitats see under Gubaidulin & Bekturganov, 1986	*Culicoides* Latreille, 1809 (13 species are listed known as hosts within and outside of Kazakhstan)	*Heleidomermis vivipara* Rubzov, 1970 / NL	Gubaidulin & Bekturganov, 2001 (a review)
Russia (NW European part: Karelia) / littoral zone of small oligotrophic lakes	Later instar larvae of *Bezzia annulipes* (Meigen, 1830) and *Palpomyia lineata* (Meigen, 1804)	*Limnomermis* Daday, 1911 (postparasitic larvae) / <<1%	Przhiboro, 2001
Russia (NW European part: Leningrad Prov.) / water edge in small oligo- and mesotrophic lakes	Later instar larvae of *Bezzia* near *winnertziana* Kieffer, 1919 (quoted as *B.* aff. *gracilis* (Winnertz, 1852)) and *Palpomyia lineata* (Meigen, 1804)	*Limnomermis* Daday, 1911 (postparasitic larvae) / <<1%	Przhiboro, 2001
Russia (southern Siberia: Gornyi Altai)	Adult females of several species of *Culicoides* Latreille, 1809, including *C. obsoletus* (Meigen, 1818) and *C. grisescens* Edwards, 1959 as abundant species	Several species of Mermithoidea (no details) / 1.4%	Glushchenko, 2002
Spain (Catalonia: Barcelona Prov.) / UV light trap set on a farm stocked with sheep and goats surrounded by typical mediterranean evergreen oak forest	Adult of *Culicoides circumscriptus* Kieffer, 1918	*Heleidomermis cataloniensis* Poinar & Sarto i Monteys, 2008 / 10%	Sarto i Monteys et al., 2003; Poinar & Sarto i Monteys, 2008
Myanmar (Early Cretaceous Burmese amber)	Adult *Atriculicoides swinhoei* (Cockerell, 1919) (fossil)	*Cretacimermis protus* Poinar & Buckley, 2006 (fossil)	Poinar & Buckley, 2006
Spain (Catalonia: Girona Prov.) / open silty mud edges of stagnant farm pond	Larva of *Culicoides circumscriptus* Kieffer, 1918	*Heleidomermis cataloniensis* Poinar & Sarto i Monteys, 2008 / to 68%	Poinar & Sarto i Monteys, 2008
Myanmar (Early Cretaceous Burmese amber)	Adult *Leptoconops rossi* Szadziewski, 2004 (fossil)	*Cretacimermis* Poinar, 2001 (fossil, as *Cretacimermis* sp.)	Poinar & Sarto i Monteys, 2008
Spain (Catalonia: Girona Province) / open, silty mud edges of stagnant farm pond	Adults and larvae of *Culicoides parroti* Kieffer, 1922	*Heleidomermis cataloniensis* Poinar & Sarto i Monteys, 2008 / NL	Sarto i Monteys, unpublished

[1] Supplement to review by Wirth [1977a].
[2] Includes the families Mermithidae and Tetradonematidae; as a rule, nematodes identified as "Mermithoidea" belong to Mermithidae.
[3] Prevalence = percentage parasitism in a collection from a particular site and time (i.e. point prevalence); NL = "not listed".
[4] *Aproctonema chapmani* (family Tetradonematidae) is the only listed nematode not in the Mermithidae.
[5] Oviparity assumed but not shown; *H. ovipara* later synonymized with *H. vivipara* [Gafurov, 1986a, 1997].
[6] Saidalieva in two publications [1985 and 1986] considered the results of the same study. Possibly, some values in Saidalieva [1986] are erroneous.

Many entomologists (see list in Table 1) did not attempt to attribute the parasite to a genus. This is due to the difficulty of classifying mermithids, even for specialists. The morphological classification of mermithids is based on adult characters, and immatures often cannot be identified. Therefore, nematologists have established provisional collective genera for immature mermithids or adult mermithids in which the diagnostic characters are obscured. The collective genus *Agamomermis* Stiles, 1903 was established for extant mermithids that could not be placed in present day genera [Poinar & Welch, 1981]; the same would apply to the genus *Ceratomermis* Rubzov, 1978. According to Poinar [personal communication] there are 36 extant, non-collective genera of the family Mermithidae. Similarly, the genera *Cretacimermis* Poinar, 2001 and *Heydenius* Taylor, 1935 were established as collective genera for mermithids from the Cretaceous and from the Tertiary, respectively; *Heydenius* had been formerly erected for fossil mermithids in general, and was later restricted to only those from the Tertiary [Poinar, 2001, 2003].

The complexity mentioned above is responsible for most early genus or species-level identifications of mermithids in ceratopogonids being either incomplete or wrong. For example, Callot [1959], in France, recorded an *Agamomermis* sp. from *Culicoides kibunensis* (as *C. cubitalis*), and Rubzov [1967] described *Agamomermis heleis* from an adult *Culicoides pulicaris* (possibly confused with the widespread *C. punctatus* — see Table 1) collected in Kazakhstan. Also, in India, Sen & Das Gupta [1958] and Das Gupta [1964] found mermithids in *Culicoides oxystoma* (as *C. alatus*) and *Atrichopogon* sp. adults respectively. Callot & Kremer [1963], in France, reported mermithids causing intersexual forms in five *Culicoides* spp. (Table 1). All these latter records identified the mermithid as a *Mermis* sp. (a highly unlikely generic attribution). Chapman et al. [1969] found in Louisiana (USA) the well-known mermithid genus *Romanomermis* parasitizing an adult of *Culicoides nanus*, which may also be an erroneous identification of the mermithid (see below). Since Rubzov [1974b], new records of extant mermithids found in adults or larvae of ceratopogonids most often have been assigned to either "Mermithidae" or to one of the four (three are now valid) described species of the genus *Heleidomermis* (see below). Table 1 summarizes these recent records.

Intersexes

Mermithid parasitism may trigger abnormal host development, morphology (including parasitic castration as discussed below), and/or behaviour. The extent of such abnormalities varies notably, even within a species. Sometimes the changes in the morphology are very obvious, such as a midge possessing male genitalia, but generally female-like antennae, mouthparts and wing proportions. In other cases the abnormalities are nearly imperceptible. In many cases, the abnormalities are produced through a change in the rate at which the male/female factors present in all animal cells are expressed; the result is then the formation of "intersexes". According to Smith & Perry [1967], intersexes are "abnormal individuals of a species in which some primary but usually secondary sexual characteristics are changed in the direction of the opposite sex but fail to reach the ultimate in development attained by a normal individual of that sex". Wigglesworth [1934] mentioned abnormal temperatures and internal parasitism as two independent factors that might cause such rate changes in insects leading to the formation of intersexes. Within Ceratopogonidae, the males of some species scattered throughout the family possess female-like antennae [Borkent, personal communication], but the underlying causes of such shifts in sexual features are unknown and likely do not originate in parasitism or abnormal temperatures.

Since not all entomologists have looked specifically for intersex characteristics in the parasitized midges they encountered (only the presence of the worms in the abdomen of the midges was mentioned), this probably contributes to the relative scarcity of intersex records of ceratopogonids. Mermithid-induced intersexes probably are more common than is suggested in the literature.

Some entomologists did notice and reported the presence of intersexes produced by mermithid parasitism (Table 1). In this paper we do not include intersex reports unless mermithids were also noted, since they can be caused by other factors. A good example is Kieffer [1914], who described *Forcipomyia heterocera* (today *F. allocera*). Kieffer mentioned two males: one with normal (male) antennae and a second one with female-like antennae. He thought this was naturally occurring intraspecific variation in males — probably this is why he named the species "*heterocera*". In fact, he did not observe mermithids or attribute the feminized antennae of the one male to parasitism. Forty years later, Thienemann [1954] assumed the Kieffer intersex specimen must have been caused by mermithid parasitism, but there was no evidence for it; the cause of the intersex condition of Kieffer's 1914 *Forcipomyia allocera* male is not known. Some naturally occurring species of *Forcipomyia* exist which have permanently feminized male antennae (basically, reduced antennal plume and the terminal 5 flagellomeres elongate); this also occurs in some other genera of Ceratopogonidae, including the closely related *Atrichopogon* [e.g., see Macfie, 1933]. Such intersexual conditions have nothing to do with mermithid parasitism.

Beck [1958] reported for the first time ceratopogonid intersexes produced by mermithid nematodes; she used the term "gynandromorphs", with female heads and male genitalia. Such specimens were found among *Culicoides haematopotus* (one specimen) and *C. crepuscularis* (several parasitized specimens) collected in light-traps in northwestern Florida, USA. Soon after, Callot [1959] near Strasbourg, France, reported intersexes in *Culicoides kibunensis* (see Table 1 for details). Callot & Kremer [1963] reported mermithid parasitism in multiple *Culicoides* species (see Table 1). They noticed that their presumably genetically male *Culicoides* intersex specimens had the maxillary palps, epipharynx, antennae and wings modified to resemble

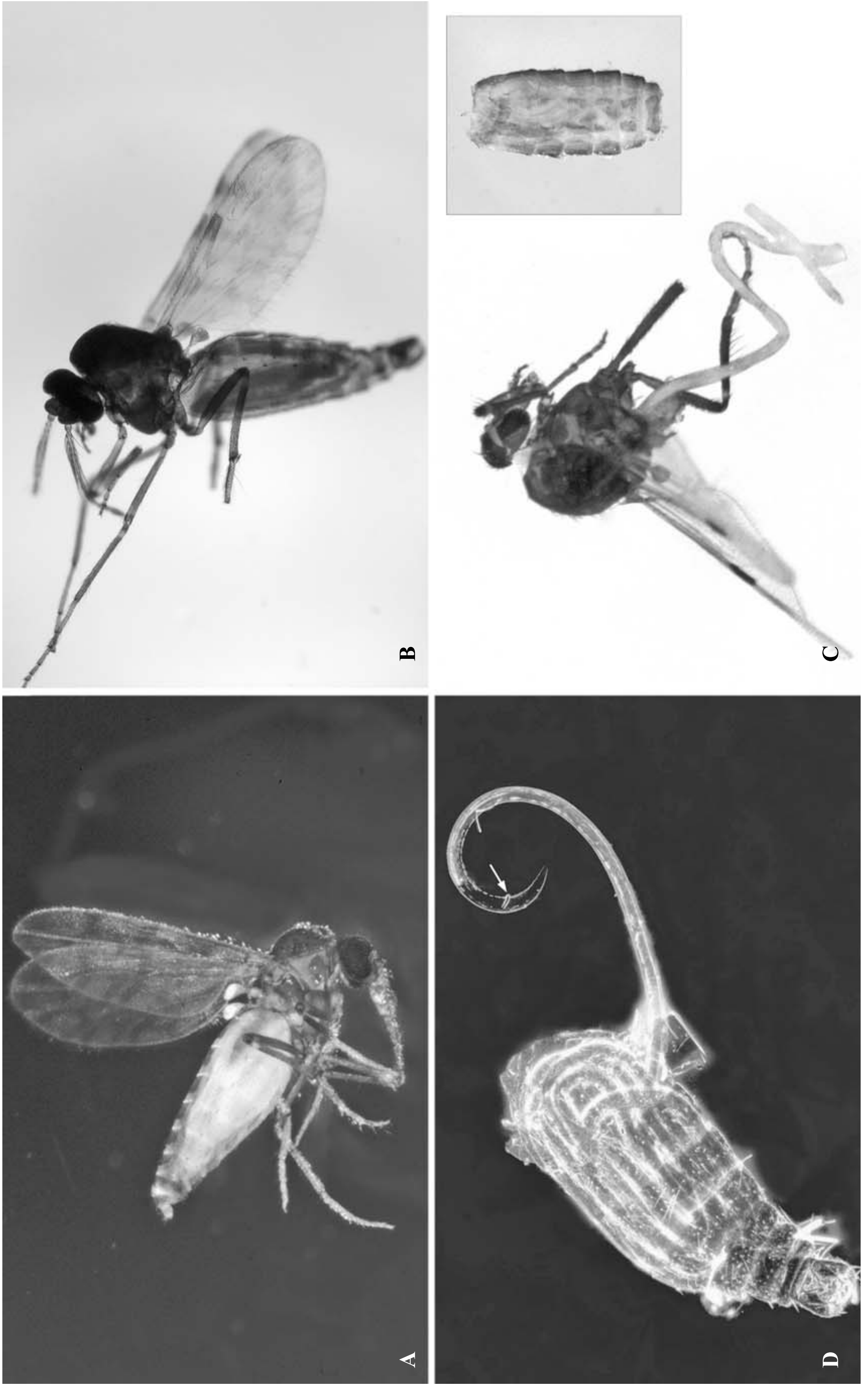


Fig. 1. *Heleidomermis cataloniensis* parasitism in adult *Culicoides circumscriptus* (female midge in A, males in B and D) and *C. parroti* (female midge in C). Note male nematode spicule (arrow) visible in D. Photos by V. Sarto i Monteys.

Рис. 1. *Heleidomermis cataloniensis*, паразитирующие в имаго *Culicoides circumscriptus* (А — самка, В и D — самцы мокрецов) и в *C. parroti* (C, самка мокреца). На рис. 1D стрелкой показана спикула самца нематоды. Фотографии В. Сарто и Монтейса.

those of females, whereas the abdomen and genitalia remained typically masculine. The parasitized females showed little sexual modification.

Sarto i Monteys et al. [2003] presented an extensive, critical review of old and new records reporting mermithid-induced intersexes in ceratopogonids. They also reported one intersex male of *C. circumscriptus* from Catalonia (Spain). It had the abdomen and external genitalia of a typical male, while the antennae resembled those of a female. Later, the mouthparts of the same specimen (parasitized by *Heleidomermis cataloniensis*) were thoroughly studied by Poinar & Sarto i Monteys [2008]. The modifications indicated (affecting the labrum-epipharynx, lacinia, mandibles, hypopharynx and maxillary palps), also partly resembled those of a female.

Because of these female traits, there has been speculation regarding whether intersexes of ceratopogonids might be able to take blood from host vertebrates, as the parasite might benefit from the added nourishment of a host blood meal [Poinar & Sarto i Monteys, 2008]. McKeever et al. [1997] employed scanning electron microscopy to study the mouthparts, antennae and external genitalia of intersexual forms of *Culicoides stellifer* parasitized by mermithid nematodes (possibly *H. magnapapula*) in Georgia, USA and compared them to typical, unparasitized specimens. The intersex specimens with male genitalia may be assumed to be genetically male, as is true for chironomids [Wülker, 1975]. Intersexes had antennae which closely resembled those of females in length of setae and number of long flagellomeres, and their mandibles were female in terms of the size and shape of teeth. However, the labrum, hypopharynx, laciniae and the average number of maxillary palpal sensilla resembled those of males, the first three structures being modified so as to be nonfunctional for imbibing blood.

Parasitic castration

Parasites commonly reduce fecundity of their invertebrate hosts, or in fact essentially castrate them [e.g. Wülker, 1964; Hurd, 2001]. This is little studied with ceratopogonids parasitized by mermithids, but would be an interesting area of study. In mermithid-parasitized *Chironomus rempeli* Thienemann, 1941, the dominant form of intersex was a basically female head but male external genitalia [Rempel, 1940], a familiar pattern with intersex ceratopogonids as well [loc. cit.]. Later work showed that such changes in chironomids were associated with severe parasitic damage to the ovaries, and to a lesser extent the testes, in the period of host development subsequent to invasion of late-stage larvae by the nematodes [Wülker, 1964, 1970, 1975].

In general, workers have reported only occasional mermithids in adult ceratopogonids. If many individuals of a particular species have harboured nematodes [e.g. Phelps & Mokry, 1976], details of parasitism or its effects have not been provided. Glukhova [1967], Mullens & Schmidtmann [1982] and Glushchenko [2002] have reported complete resorption and/or degeneration of oocytes as a result of mermithid parasitism in *Leptoconops* sp., *Culicoides variipennis* and *C. obsoletus*, respectively. However, in Glushchenko [2002], the resorption of oocytes in most parasitized *Culicoides* females was only partial.

The study of Jeu [1977] is unique in reporting details of mermithid parasitism among 152 naturally blood-engorged, parasitized *C. riethi* collected from southern China. The females were dissected to count the nematodes and examine ovarian development relative to stages of blood digestion. Of 7,936 engorged females dissected, 152 (1.9%) harboured nematodes. Of those parasitized, 47% harboured a single mermithid, 17% had two nematodes, 13% had three, 9% had four, 7% had five, 2% had six, 4% had seven, 0.7% had eight, and 0.7% harboured a remarkable ten nematodes. Three parasitized females (1–2 nematodes each) also had fully developed eggs (Christopher's stage 5). However, the large majority of parasitized females (98%) were not fully gravid, and 48% showed signs of irregular ovarian development and/or follicle resorption. While the identity of the mermithid is unknown, the paper is the only substantial study that shows that some parasitized females can in fact ingest blood and develop eggs, although oogenesis is substantially impaired by parasitism.

Fossil mermithids parasitising the Ceratopogonidae

Mermithid parasitic associations with the Ceratopogonidae date at least to the Early Cretaceous. Poinar [1983] speculated that the Mermithidae arose in the Triassic from microbotrophic members of the Dorylaimida. The first description of a fossil mermithid parasitizing a ceratopogonid — a female tentatively identified as *Atriculicoides swinhoei* — was reported by Poinar & Buckley [2006] from Early Cretaceous Burmese amber dated at 100–110 million years ago. This mermithid was named *Cretacimermis protus*. Soon after, and from the same Burmese amber, Poinar & Sarto i Monteys [2008] reported another ceratopogonid (also a female), identified as *Leptoconops rossi*, parasitized by two conspecific mermithids, which were assigned to the genus *Cretacimermis*. They were not given a specific name, since the obscured diagnostic characters made it unclear whether those were conspecific with the previously described *C. protus.*

Another described fossil mermithid from Lebanese amber from the Cretaceous period was initially given the name *Heleidomermis libani* [Poinar et al., 1994], since the host was formerly thought to be a ceratopogonid (the genus *Heleidomermis* is restricted to ceratopogonids). However, it was later found to be a chironomid [Borkent, 2000] and therefore reallocated into the provisional genus *Cretacimermis*, a collective genus for Cretaceous material [Poinar, 2001].

Mermithids in larval Ceratopogonidae: A brief review

Most of the published data on mermithid parasitism in immatures of Ceratopogonidae refer explicitly or

very likely to members of the genus *Heleidomermis*. These primarily are parasites of bloodsucking midges of the genus *Culicoides* (see Table 1), and those data will be discussed in later parts of this paper.

There are relatively few publications that note parasitism of other genera of Ceratopogonidae by mermithids. In addition to the adult cases listed earlier, unidentified mermithids from larvae of several ceratopogonid taxa have been reported. They include mermithids from *Dasyhelea flavifrons* (as *D. obscura*) in England [Keilin, 1921], *Dasyhelea* sp. in France [Callot & Kremer, 1963], *Leptoconops* sp. in Azerbaijan [Glukhova, 1967], and *Palpomyia* sp. in Leningrad Province, the European USSR [Glukhova, 1979]. Rubzov [1974b] described two new mermithid species as mature postparasitic larvae, which emerged from larvae of Ceratopogonidae collected by Glukhova in Pskov Province, the European USSR. The first species, *Gastromermis bezzii*, was reared from *Bezzia* sp., and the second one, *Agamomermis gluchovae*, was reared from an unidentified ceratopogonid larva. Judging from the figure in Rubzov [1974b: Fig. 1A on p. 214], the host of *A. glukhovae* also belongs to the Palpomyiinae. *Heleidomermis vivipara*, the species reared mostly from *Culicoides*, was recorded also from larvae of "non-bloodsucking Ceratopogonidae" in Uzbekistan [Lebedeva, 1988; Lebedeva & Gafurov, 1994]. Recently, the mermithid genus *Limnomermis* was recorded as postparasitic larvae emerged from later instar larvae of three Palpomyiinae species in Leningrad Province and Karelia, northwestern Russia [Przhiboro, 2001; see Table 1].

Four genera of Mermithidae other than *Heleidomermis* have been recorded from immature *Culicoides*: *Agamomermis*, *Ceratomermis*, *Romanomermis* and *Spiculimermis* (see Table 1). Additionally, the mermithids in many papers were undetermined or referred to as "*Mermis*" [Dzhafarov, 1962, 1964; Glukhova, 1967, 1979; Chapman et al., 1968; Mirzaeva, 1971, 1989; Trukhan, 1975; McKeever et al., 1997; Glushchenko, 2002]. Aside from Mermithidae, one representative of the family Tetradonematidae, *Aproctonema chapmani*, was recorded from *Culicoides* [Nickle, 1969; Chapman et al., 1969; Chapman, 1973].

In total, mermithid parasitism has been recorded from 40 species of *Culicoides* belonging to 11 subgenera (see Table 2). In 23 of these species, mermithids were observed as parasites of larvae, while adult parasitism has been noted in 27 species. Immature *Culicoides* are parasitized by mermithids in a wide range of shallow aquatic and semiaquatic habitats. Among them are tree holes, marshes and *Sphagnum* bogs, the shorelines of different types of rivers and lakes, and numerous small, shallow bodies of mostly standing water, which are very diverse in the conditions (see Table 1).

Keeping in mind the earlier concerns regarding the validity of some genera, one genus of Tetradonematidae (*Aproctonema* Keilin, 1917) and seven genera of Mermithidae have been reported from Ceratopogonidae: *Agamomermis* Stiles, 1903, *Ceratomermis* Rubzov, 1978, *Gastromermis* Micoletzky, 1923, *Heleidomermis* Rubzov, 1970, *Limnomermis* Daday, 1911, *Romanomermis* Coman, 1961, and *Spiculimermis* Artyukhovsky, 1963. As mentioned above, two of these are collective genera, *Agamomermis* and *Ceratomermis*, and sometimes regarded as doubtful. The fifteen species of *Romanomermis* exclusively parasitize Culicidae, except for the puzzling type specimen from an amphipod [Platzer, 2007]. The finding from *Culicoides* [Chapman et al., 1969; Chapman, 1973] thus is suspect and requires confirmation. An eighth described fossil genus, *Cretacimermis*, also has been reported from adult ceratopogonids imbedded in amber [Poinar, 2001].

Geographical distribution of mermithids in Ceratopogonidae

Figure 2 presents the known geographic distribution of mermithids found in either adults or larvae of Ceratopogonidae worldwide (see also Tables 1 and 2). Clearly, mermithid parasitism of *Culicoides* is a fairly widespread phenomenon, including the Palaearctic, Nearctic, Oriental and Afrotropical zones. In the Palaearctic, mermithid parasitism of *Culicoides* has been recorded



Fig. 2. Known distribution of mermithids in Ceratopogonidae (adults and/or larvae) in North America (top) and Eurasia (bottom). Zimbabwe record not shown (see Table 1).

Рис. 2. География известных к настоящему времени находок Mermithida в Ceratopogonidae (в имаго и/или в личинках), в Северной Америке (вверху) и в Евразии (внизу). Находка из Зимбабве на карте не показана (см. Таблицу 1).

Table 2. Species of *Culicoides* Latreille, 1809, parasitized by Mermithida.
Таблица 2. Виды рода *Culicoides* Latreille, 1809, на которых паразитируют представители отряда Mermithida.

Species of *Culicoides*	Synonyms used [1]	Subgenus [2]	Regions [3]	Host stages parasitized[4]	Mermithids [5]
C. alazanicus Dzhafarov, 1961	*C. musilator* Kremer & Callot, 1961	*Oecacta* Poey, 1853 [6]	France	Adult	NI
C. arboricola Root & Hoffman, 1937		*Oecacta*	USA (Louisiana)	Larva	*Aproctonema chapmani* Nickle, 1969
C. circumscriptus Kieffer, 1918		*Beltranmyia* Vargas, 1953	Russia (Buryatia), Uzbekistan, SE Kazakhstan, Spain	Larva, adult	*Heleidomermis vivipara* Rubzov, 1970, *H. cataloniensis* Poinar & Sarto i Monteys, 2008
C. crepuscularis Malloch, 1915		*Beltranmyia*	USA (Florida)	Adult	NI
C. dendrophilus Amossova, 1957		*Amossovia* Glukhova, 1989	Russia (Primorie)	Larva	NI
C. desertorum Gutsevich, 1959		*Beltranmyia*	Uzbekistan, SE Kazakhstan	Larva, adult	*H. vivipara*
C. fascipennis (Staeger, 1839)		*Silvaticulicoides* Glukhova, 1977	France, Russia (Buryatia)	Larva, adult	NI
C. festivipennis Kieffer, 1914	*C. odibilis* Austen, 1921	*Oecacta* [6]	France, Russia (Buryatia), SE Kazakhstan	Larva, adult	NI, *H. vivipara*, *Ceratomermis heleis* (Rubzov, 1967)
C. grisescens Edwards, 1939		*Culicoides* Latreille, 1809	Russia (Karelia, Buryatia, Gornyi Altai)	Larva, adult	NI
C. haematopotus Malloch, 1915		*Diphaomyia* Vargas, 1960	USA (Florida)	Adult	NI
C. helveticus Callot, Kremer & Deduit, 1962		*Monoculicoides* Khalaf, 1954	Russia (Buryatia)	Larva	*H. vivipara*
C. inornatipennis Carter, Ingram & Macfie, 1920		*Wirthomyia* Vargas, 1973	Zimbabwe	Adult	NI
C. jacobsoni Macfie, 1934	*C. buckleyi* Macfie, 1937	*Avaritia* Fox, 1955	Malaysia	Adult	NI
C. kibunensis Tokunaga, 1937	*C. cubitalis* Edwards, 1939	*Oecacta* [6]	France	Adult	NI
C. leucostictus Kieffer, 1911	*C. praetermissus* Carter, Ingram & Macfie, 1920, *C. pycnostictus* Ingram & Macfie, 1925, *C. ravus* de Meillon, 1936	*Beltranmyia*	Zimbabwe	Adult	NI
C. manchuriensis Tokunaga, 1941		*Beltranmyia*	Russia (Buryatia)	Larva	NI, *H. vivipara*
C. nanus Root & Hoffman, 1937		*Oecacta*	USA (Louisiana)	Larva	*Romanomermis* sp., NI
C. nubeculosus (Meigen, 1830)		*Monoculicoides*	Russia (Karelia)	Larva	NI, *H. vivipara*
C. obscurus Tokunaga & Murachi, 1959	*C. pungens* de Meijere, 1909	*Avaritia*	Malaysia	Adult	NI
C. obsoletus (Meigen, 1818)		*Avaritia*	France, England, Belorussia, Russia (Gornyi Altai)	Adult	NI

Species of *Culicoides*	Synonyms used [1]	Subgenus [2]	Regions [3]	Host stages parasitized[4]	Mermithids [5]
C. occidentalis Wirth & Jones, 1957	*C. variipennis* (Coquillett, 1901)	*Monoculicoides*	USA (Virginia)	Larva	NI
C. odiatus Austen, 1921	*C. lailae* Khalaf, 1961	*Oecacta* [6]	Uzbekistan	Larva	*H. vivipara*
C. orientalis Macfie, 1932		*Avaritia*	Malaysia	Adult	NI
C. oxystoma Kieffer, 1910	*C. alatus* Das Gupta & Ghosh, 1956	*schultzei*-group (*Remmia* Glukhova, 1977, according to Glukhova, 1989)	Malaysia, India	Adult	NI
C. parroti Kieffer, 1922		*Monoculicoides*	Spain	Larva, adult	*H. cataloniensis*
C. peregrinus Kieffer, 1910		*Hoffmania* Fox, 1948	Malaysia	Adult	NI
C. pictipennis (Staeger, 1839)		*Oecacta* [6]	England	Adult	NI
C. pulicaris (Linnaeus, 1758)		*Culicoides*	Russia (Buryatia)	Larva	NI, *H. vivipara*
C. pulicaris [probably *C. punctatus* (Meigen, 1804)]		*Culicoides*	SE Kazakhstan	Adult	*C. heleis*
C. punctatus (Meigen, 1804)		*Culicoides*	Uzbekistan	Larva	*H. vivipara*
C. puncticollis (Becker, 1903)		*Monoculicoides*	Russia (Karelia), Uzbekistan, SE Kazakhstan	Larva, adult	NI, *H. vivipara*, *Agamomermis* spp.
C. riethi Kieffer, 1914		*Monoculicoides*	Russia (Khabarovsk Terr.), SE Kazakhstan, China (Chungking)	Larva, adult	NI, *H. vivipara*
C. salinarius Kieffer, 1914		*Beltranmyia*	Uzbekistan	Larva	*H. vivipara*
C. shortti Smith & Swaminath, 1932		unplaced	Malaysia	Adult	NI
C. sibiricus Mirzaeva, 1964		*Beltranmyia*	Russia (Buryatia)	Larva	NI
C. sonorensis Wirth & Jones, 1957	*C. variipennis* (Coquillett, 1901)	*Monoculicoides*	USA (California)	Larva, adult	*H. magnapapula* Poinar & Mullens, 1987
C. sphagnumensis Williams, 1955		*Beltranmyia*	Russia (Buryatia)	Larva	NI
C. stellifer (Coquillett, 1901)		*Oecacta*	USA (Florida, Georgia)	Adult	NI (possibly, *H. magnapapula* in Georgia)
C. stigma (Meigen, 1818)		*Monoculicoides*	Russia (Karelia)	Larva	NI, *H. vivipara*
C. tropicalis Kieffer, 1913	*C. babrius* de Meillon, 1943	*Diphaomyia*	Zimbabwe	Adult	NI
C. variipennis (Coquillett, 1901)		*Monoculicoides*	USA (Alabama, New York)	Larva, adult	*H. magnapapula* (not certain in Alabama)

NOTE. In this table, we summarize briefly the data on mermithid parasitism in field-collected *Culicoides* with reliable species identifications of hosts. For details, see Table 1.

1 Incorrect identifications not included (see also Table 1 and next).
2 Subgenera of *Culicoides* according to Glukhova [1989] (Palaearctic Region), Blanton & Wirth [1979] (Nearctic Region), and Wirth & Hubert [1989] (Oriental Region). Following Khamala & Kettle [1971], subgenera are not recognized among Afrotropical *Culicoides*, so those tentatively have been placed according to the above-cited works. Lacking a full cladistic analysis, subgeneric placement is subject to later modification.
3 Regions and countries of the former USSR according to present-day delimitation.
4 Larval and adult stages were included.
5 NI = "not identified"; *Heleidomermis ovipara* is considered a synonym of *H. vivipara*.
6 The listed species of *Oecacta* are categorized as unplaced by Szadziewski & Borkent [2004].

from northern boreal forests (62°N, Karelia) to southern desert environments (about 40°N, Uzbekistan), from Spain and Britain in the West to the Far East (Khabarovsk and Primorie territories of Russia), and in montane regions up to 1000 m above sea level [Saidalieva, 1985]. Where mermithids have not been reported from ceratopogonids, this is likely due to lack of investigation, as is true for the geographical centre of North America. Mermithid parasitism no doubt eventually will be shown to be quite common globally, and it is almost certain that more species exist than are currently described.

The genus *Heleidomermis*

Using mermithid specimens reared by Glukhova from *Culicoides* larvae in Russian Karelia (northwestern European USSR near Finland), Rubzov described a new genus, *Heleidomermis*, with the type species *H. vivipara* Rubzov [Rubzov, 1970]. Later on, *H. vivipara* was found also in Kazakhstan and Uzbekistan (see Table 1 for citations). *Heleidomermis* was named after the family of biting midges (formerly Heleidae and now Ceratopogonidae) that were the hosts of the nematodes. The species name, *vivipara*, was given due to the highly unusual method of reproduction. The vast majority of mermithids, including the familiar *Romanomermis* spp. useful in mosquito biological control, emerge from their invertebrate hosts as immatures (postparasitic juveniles); only later do they moult to the adult, mate, and lay eggs in the environment [Rubzov, 1974a; Platzer et al., 2005]. The strange *H. vivipara*, on the other hand, moulted to the adult stage in the host, and emerged from the host ready to mate immediately in the wet habitat mud. Also very unusual was the fact that the fertilized eggs hatched within the body of the adult female mermithid (ovoviviparous), and the preparasitic (infective) juveniles were expelled into the environment ready to infect a host larva immediately.

A second described species of *Heleidomermis*, *H. ovipara* Rubzov, was reared by Mirzaeva from *Culicoides* larvae collected in the Tunkin Valley in Buryatia, USSR [Rubzov, 1974b]. As the name implies, this second species was thought to be oviparous. Rubzov [1974b] included this feature in the diagnosis of the new species, along with some morphological distinctions of *H. vivipara* from Karelia, and this fundamental putative biological difference was very important in separating *H. ovipara* from *H. vivipara*. However, from the brief discussion by Rubzov [1974b] and also from data in Mirzaeva [1971] it appears that these mermithids were fixed shortly after emergence from the host. Thus, the critical aspect of deposition of fertile eggs by live females (i.e. oviparity) actually was not observed. Gafurov [1986a, 1986b, 1997] synonymized *H. ovipara* with *H. vivipara*. Although Gafurov did not comment on this synonymy in detail, he studied a large number of *Heleidomermis* specimens from Middle Asia, analysed their development, and probably examined Rubzov's type specimens deposited in the Zoological Institute (St.Petersburg). We accept that synonymy.

The second valid species was initially reported as *H. vivipara* from larvae of a different *Culicoides* host species, *C. variipennis*, which was many thousands of kilometres away and on a different continent, in New York State, USA [Mullens & Rutz, 1982]. While the biology, and particularly the reproduction, are similar to *H. vivipara*, the New York nematode (found also in California) was later recognized as a distinct species, *H. magnapapula* Poinar & Mullens [Poinar & Mullens, 1987]. Both quantitative and qualitative morphological characters, such as shape and size of amphidial pouches or egg diameter, separate the two species.

A third valid species in the genus was described from larvae of *C. circumscriptus* in western Europe (the Catalonian region of northeastern Spain) as *H. cataloniensis* Poinar & Sarto i Monteys [Poinar & Sarto i Monteys, 2008]. Being so recently discovered, little is known of its life history thus far, but the biology appears to be similar to *H. vivipara* and *H. magnapapula* [Poinar & Sarto i Monteys, 2008].

All known *Heleidomermis* spp. (*H. vivipara*, *H. magnapapula* and *H. cataloniensis*) thus emerge from the host as adults, have eggs that hatch internally following mating, and produce preparasitic, infective juveniles directly from the female's body into the environment. Based on the complex of advanced morphological characters and the very unusual life history characteristics, Artyukhovsky [1990] erected a new subfamily for this genus, and Gafurov [1997] considered Heleidomermithinae the mostly advanced and highly specialized group in the family Mermithidae.

Heleidomermis life stages

Heleidomermis life stages are illustrated in Figs 3A–E. The relatively large and opaque adult females can sometimes be seen through the host cuticle even before emergence (Fig. 3A). Males (Fig. 3B on right) are considerably thinner, often are shorter in length than females, and possess paired spicules (intromissive organs used in mating). Size and shape of the spicules, as well as size and shape of amphidial pouches or cephalic papillae in both sexes, are useful diagnostic characters. Shortly after emergence from the host larvae (Fig. 3C), adult *H. magnapapula* have a very strong urge to form tight clusters in dishes of water (Fig. 3D). This presumably is for mating, although it is not known if they do that in natural mud habitats or how they respond to substrate structure. After several hours to a day they lose this urge to cluster and disperse more loosely in the dishes.

The size of adults varies within a species and possibly reflects host species and/or body size as well. For example, *H. magnapapula* females from the relatively large North American species *C. sonorensis* range from 6–15 mm in length. *H. cataloniensis*, on the other hand, utilizes smaller *Culicoides* spp. such as *C. circumscriptus*, and their much smaller females typically range from about 3–8 mm [Poinar & Sarto i Monteys, 2008].

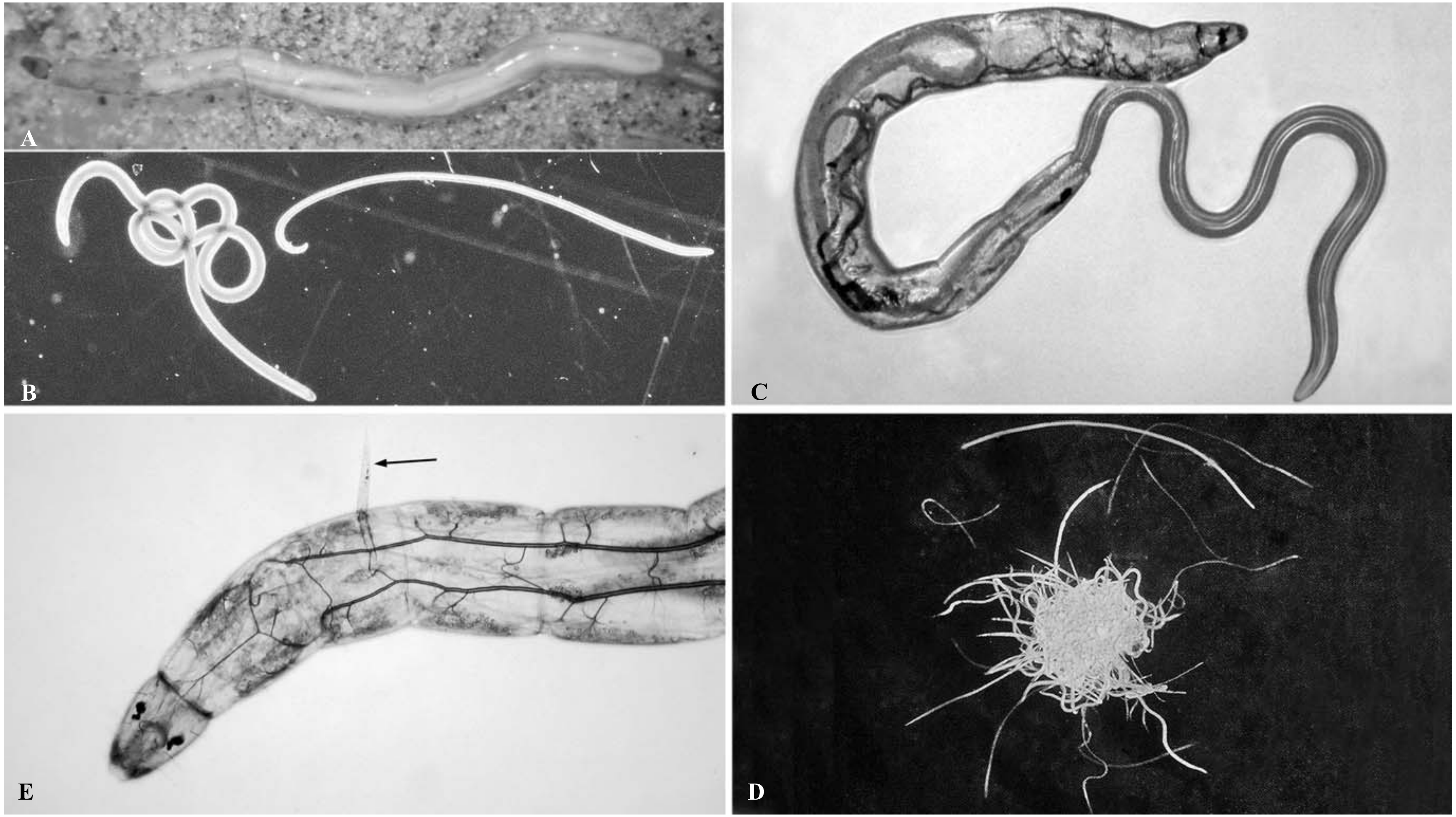


Fig. 3. Life stages of *Heleidomermis*. A — adult female *H. magnapapula* within a 4th instar *Culicoides variipennis* from New York State, USA; B — adult *H. magnapapula* female (left) and male (right) after emergence from host larvae; C — adult *H. magnapapula* female emerging from *C. sonorensis* in California, USA; D — cluster of mating *H. magnapapula* males and females shortly after emergence from their host larvae; E — preparasite of *H. cataloniensis* penetrating the cuticle (see arrow) of a 3rd instar larva of what probably is *C. circumscriptus*. Photo B by Jack Kelly Clark, University of California, Davis. Other photos by B. Mullens.

Рис. 3. Стадии жизненного цикла *Heleidomermis*. A — взрослая самка *H. magnapapula* в личинке *Culicoides variipennis* 4-го возраста из штата Нью-Йорк, США; B — взрослые особи *H. magnapapula* после выхода из личинок хозяина: самка (слева) и самец (справа); C — взрослая самка *H. magnapapula* в момент выхода из личинки *C. sonorensis*, собранной в Калифорнии, США; D — клубок самцов и самок *H. magnapapula*, спаривающихся вскоре после выхода из личинок мокрецов; E — инвазионная личинка (препаразит) *H. cataloniensis*, проникающая в хозяина (вероятно, в личинку *C. circumscriptus* 3-го возраста) через кутикулу (указано стрелкой). Автор фотографии B — Джек Келли Кларк (Калифорнийский Университет, Дэвис); автор остальных фотографий — Б. Мюлленс.

In *H. vivipara*, the size of adult females varies very substantially with the locality and probably host size: 5.2–6.8 mm in Russian Karelia [Rubzov, 1970], 4.3–8.2 mm in Uzbekistan [Gafurov & Saidalieva, 1984], and 9.9–15.4 mm in SE Kazakhstan [Gubaidulin & Bekturganov, 1986].

Eggs in *H. vivipara* [Rubzov, 1970], *H. magnapapula* and *H. cataloniensis* [Poinar & Sarto i Monteys, 2008] hatch internally a few days after mating. Depending on size, each female may produce from about 500–2,500 progeny [e.g. Mullens & Velten, 1994; Sarto i Monteys, unpublished data]. Eggs contain the L1 stage mermithid larvae, which moult once within the egg and emerge as L2 stage larvae. This infective preparasite stage (Fig. 3E) possesses a stylet to aid in penetration of the host cuticle and direct entry into the host hemocoel. Entrance of preparasites via the host oral cavity also has been mentioned for *H. vivipara* [Bekturganov, 1991], but this might be intentional ingestion of the preparasites by the host as food items. Preparasites of *H. magnapapula* which are eaten by larvae of *C. sonorensis* are damaged or killed and are not thought to be viable [B. Mullens, unpublished observation]. Two intermediate immature growth stages (L3 and L4) and the final moult to the adult occur within the host. Normally, adult nematodes leave the host and copulate in the host habitat. While it may be possible for mermithids to mate within a host (provided both sexes are there), it is unknown whether they actually do this, and in fact it is fairly uncommon for a single host to contain both sexes of at least *H. magnapapula*. About 5–6% either of field-collected, or laboratory-infected, parasitized *C. sonorensis* contained both sexes of that species [Paine & Mullens, 1994; Mullens & Velten, 1994b]. Lebedeva [1988] also reported on solitary *Culicoides* larvae with numerous *H. vivipara* inside, both males and females; Rubzov [1974b] noted a male and a female *H. vivipara* (as *H. ovipara*) inside the same host larva.

Comparative biology of *Heleidomermis magnapapula* and *H. vivipara*

Due partially to its *in vivo* colonization (see below), the most thoroughly studied *Heleidomermis* species is *H. magnapapula*, which naturally parasitizes two closely related midges in the genus *Monoculicoides — C. variipennis* in New York and *C. sonorensis* in California. Because *C. sonorensis* is a primary vector of bluetongue viruses to domestic ruminants (sheep, cattle) in the western and southern United States, the nematode is of particular interest as a potential biological control agent. The biology of this species provides a useful preliminary model for other members of the genus. Additionally, field observations and some related laboratory manipulations have been done with *H. vivipara* in four regions of the former USSR: Karelia, Buryatia, and especially in Uzbekistan and southeastern Kazakhstan (see Tables 1 and 2). Most of those publications lack detailed data, making some aspects of their interpretation difficult. In combination, however, they offer some important insights into the biology and distribution of *H. vivipara*, and this allows some comparisons to be made between the two *Heleidomermis* species.

Geographical distribution and levels of parasitism

Heleidomermis magnapapula has been found in multiple habitats in New York State in larvae of *C. variipennis* [Mullens & Rutz, 1982] and in California in larvae of *C. sonorensis* [Paine & Mullens, 1994]. Reports of *Heleidomermis* parasitism in larvae of this group of midges from central Alabama [Hribar & Murphree, 1987] may be *H. magnapapula*, although this has not been determined with certainty. A few mermithid-parasitized larvae of *C. occidentalis* have been found in rather saline (6–20 g/l) mud in southwestern Virginia, USA [J. Vaughan, personal communication]; the high habitat salinity may limit parasitism. The exact identity of these nematodes is not known, but they may well be *H. magnapapula*.

If quantified, parasitism usually has been determined by holding late-stage host *Culicoides* larvae in water, either individually or collectively. However, many factors, such as host maturity at time of parasitism or collection, might influence successful parasite emergence. An alternative, and more definitive, way of assessing parasitism is to dissect the host larvae [Mullens & Luhring, 1998]. Host dissection was also used to estimate the level of parasitism by *H. vivipara* [Lebedeva, 1988, 1993; Lebedeva & Gafurov, 1994; Bekturganov, 1991].

Four mermithid sites were found in New York State; Mullens & Rutz [1982] reported average parasitism of *C. variipennis* larvae was 9.8% and 7.1% at two sites where hosts were repeatedly sampled, with individual collections ranging from 0–54% parasitism. In a more extensive survey in California, the distribution of the nematode in larvae of *C. sonorensis* was quite uneven [Paine & Mullens, 1994]. *H. magnapapula* emerged from hosts in only 25% of field populations examined (15 out of 60), although many of those populations were sampled only once, and numbers of hosts held varied [Paine & Mullens, 1994]. Levels of reported parasitism in California also vary widely within the same habitat through time. Larval *C. sonorensis* parasitism of 0–69% was observed within a single southern California dairy wastewater habitat repeatedly sampled over a period of months [Paine & Mullens, 1994].

In comparison, *H. vivipara* was found in Uzbekistan in three provinces of the four studied, but only in 6 of 82 bodies of water examined; we assume that those 82 habitats all yielded potential *Culicoides* host larvae for parasitism estimation [Lebedeva, 1988; Lebedeva & Gafurov, 1994]. In general, in other studies of *H. vivipara* (see Table 1), the parasites also were found from only some of the host larval habitats. The percentage of host larvae parasitised by *H. vivipara* in Uzbekistan has varied markedly, ranging from solitary larvae to 67% (see Table 1). The two *Heleidomermis* species thus share a patchy spatial distribution, and the maxi-

mum field larval parasitism (based on emergence) is remarkably close for the two species. Interestingly, the maximum emergence-based parasitism reported for *H. cataloniensis* also is in this exact range [68%; Poinar & Sarto i Monteys, 2008].

For both *H. magnapapula* and *H. vivipara*, the variable number of mermithids per host individual (intensity of parasitism) probably depends on the relative abundance of preparasitic mermithid larvae and their hosts. For example, no more than 1–2 *H. vivipara* per host larva were found in all habitats of SE Kazakhstan [Gubaidulin & Bekturganov, 1986; Bekturganov, 1991], and in some localities of Uzbekistan [Saidalieva, 1985]. This was also the case with *H. magnapapula* parasitism of *C. variipennis* in New York State, where 59% of all emerging nematodes were females (overall sex ratio was 0.7 males per female). In contrast, in other Uzbekistan localities about 40% of parasitized host larvae contained 3 or more parasites, and more than ten *H. vivipara* in one dissected larva were quite common [Lebedeva, 1988; Lebedeva & Gafurov, 1994]. Three or more nematodes emerging per host were fairly common in southern California; 16% of parasitized hosts yielded 3 or more males each (maximum 8 males in one host), with a sex ratio of 2.4 males per female [Paine & Mullens, 1994].

Habitat characteristics and laboratory rearing

In North America, both *C. variipennis* and *C. sonorensis* are members of the subgenus *Monoculicoides*, and are stereotypically most abundant in a distinctive type of habitat [Schmidtmann et al., 1983; Mullens, 1989]. Such habitats have shallow, standing or slowly-moving water which is polluted by animal feces and urine, and is often accessed by livestock. The mud at the edges is frequently free of vegetation (exposed, fine sediment beds) and has very gentle edge slopes (Figs 4A–C). In such habitats, larvae of *C. sonorensis* can be present at densities of several hundred or more per 30 ml of surface, edge mud [Mullens & Lii, 1987]. This also seems to be the ideal habitat for *H. magnapapula*. Interestingly, *H. vivipara* has been described from habitats that resemble this generally (Table 1). Extensive field studies in Middle Asia revealed that *H. vivipara* is abundant in shallow, semi-permanent, sunlit bodies of water. These are stagnant or slowly flowing, often muddy, brackish or/and polluted by organic material [Lebedeva, 1988; Bekturganov, 1991; Lebedeva & Gafurov, 1994]. The single known site for *H. cataloniensis* in Spain also structurally is extremely similar (Fig. 4D) to sites used by *H. magnapapula* in the USA (Fig. 4B) and *H. vivipara* in Asia.

The two free-living stages of the nematode are the infective preparasites and the adults. Only these stages would directly encounter salts etc. typical of the host habitat, while the other stages are protected within the host body. *H. magnapapula* thrives in polluted mud (up to 12% cattle manure) and moderate salinity levels (up to 150 mM NaCl), and overall survival is best at 10–50 mM NaCl, mirroring levels normally found in the host habitat [Mullens, 1989; Mullens & Luhring, 1996]. The relatively short-lived preparasites are less tolerant of higher salinities and manure pollution than the adult nematodes are, and the preparasites in particular are harmed by water lacking salts (distilled water). According to Lebedeva & Gafurov [1994], total hardness (concentration of Ca and Mg ions in water) in six field *H. vivipara* habitats was 1.1–8.6 mg equivalent per litre, which corresponded to 22–172 mg/l of Ca^{2+} and indicated a range from soft to very hard (possibly, mineralized) water.

Only a few species of *Culicoides* have been successfully colonized in the laboratory, but the host of *H. magnapapula* (*C. sonorensis*) is one of those [Hunt et al., 1999]. This is of course a huge advantage in studying aspects of the host-parasite relationship and the biology of the mermithid. In this case the host larvae are reared in pans of nutrient-rich water, with polyester pads serving as substrate for the *C. sonorensis* larvae. The mermithids thus can be reared *in vivo* in these pans also [Mullens & Velten, 1994b; Luhring & Mullens, 1997]. Briefly, adult nematodes emerge from groups of previously parasitized, 4th instar hosts held in Petri dishes of tap water, and newly-emerged mermithids mate immediately. The eggs require 3–4 days to hatch internally at 23°C, and gravid female *H. magnapapula* (almost ready to produce preparasites) are added to the *Culicoides* rearing pans when the hosts are in the late second or early third instar. While some individual females can reach 15 mm in length, female *H. magnapapula* in the laboratory usually range from 7–13 mm, and fecundity is related to size. A female 8–9 mm long produces approximately 900–1,000 preparasites. The rearing system was refined to yield 22.5% parasitism (as measured by successful nematode emergence) of host larvae by adding one gravid female (900 preparasites) per 650–700 *C. sonorensis* larvae. Nine days after adding the gravid female nematodes to the host rearing pans, the live, 4th instar larvae are sieved from the pads and can be held together in dishes for nematode emergence.

Sex ratio determination

Further improvements in the *in vivo* rearing system no doubt are possible, but higher levels of parasitism tend to come at the cost of reduced female nematode numbers and fitness (size). This is due to the characteristic sex determination mechanism of mermithids [e.g. Poinar, 1975; Petersen, 1985; Artyukhovsky, 1990; Kharchenko, 1999]. *Culicoides sonorensis* larvae with a single nematode produce female nematodes about 60–80% of the time [Paine & Mullens, 1994; Mullens & Velten, 1994b]. However, hosts that harbour two nematodes produce mostly males, and no females are produced if three or more nematodes occupy a host. This elegant feedback system allows *H. magnapapula* to track its host resources quite effectively in time. If there are few nematodes relative to the available hosts, relatively more hosts will have only a single parasite, and those will develop into females for the next generation. If, on the other hand, there are many nematodes for the available hosts in a field setting, many hosts will be



Fig. 4. Habitats of *Heleidomermis* spp.: A — livestock pond in New York State inhabited by *Culicoides variipennis* and *H. magnapapula*; B — shore zone of livestock pond inhabited by *C. variipennis* and *H. magnapapula*; C — southern California dairy wastewater pond inhabited by *C. sonorensis* and *H. magnapapula*; D — shore zone of farm pond in Catalonia (Spain) inhabited by *H. cataloniensis* and several *Culicoides* species such as *C. circumscriptus*, *C. festivipennis* and *C. parroti*. Photos by B. Mullens.

Рис. 4. Биотопы *Heleidomermis* spp.: A — пруд в штате Нью-Йорк, используемый для водопоя скота — биотоп *Culicoides variipennis* и *H. magnapapula*; B — прибрежная зона пруда, служащая биотопом развития *C. variipennis* и *H. magnapapula*; C — пруд в южной Калифорнии, в который поступают стоки от молочной фермы — биотоп *C. sonorensis* и *H. magnapapula*; D — прибрежная зона пруда у фермы в Каталонии (Испания) — биотоп *H. cataloniensis* и нескольких видов *Culicoides* — *C. circumscriptus*, *C. festivipennis* и *C. parroti*. Фотографии Б. Мюлленса.

attacked by multiple nematodes, leading to production of males and a consequent drop in reproductive capability of the population in the next generation.

Rubzov [1974b] noted the same tendency in *H. vivipara* (as *H. ovipara*), and a similar pattern was observed by Lebedeva [1988] for *H. vivipara* in Uzbekistan. Using dissections of *Culicoides* larvae, one site had a very high level of parasitism (up to 27 mermithids in one host larva, and about 50% of infested larvae containing more than one mermithid). It is unlikely a host larva could support the development of 27 mermithids to adulthood. Lebedeva reported on the predominance of males in all larvae infested by numerous mermithids, and the sex ratio (male : female) was 3 : 1.

Field distribution

A detailed field distribution study of *H. magnapapula* was done in southern California [Mullens & Luhring, 1998]. *H. magnapapula* is typically a parasite of larval *C. sonorensis*; the preparasites attack younger stage larvae (first and second instars) and emerge from the fourth instar larvae. Because of host death due to mermithid parasitism, the most accurate parasitism estimates were derived by dissecting third instar hosts. These were past the stage of peak parasitism, but nematodes had not yet killed the host. These studies showed that assessing *H. magnapapula* parasitism by waiting for them to emerge from fourth instar hosts probably underestimated true parasite impact by as much as 50%.

Those same field studies [Mullens & Luhring, 1998] demonstrated that free-living (postparasitic) adult nematodes were most abundant above waterline, where the early-stage hosts were parasitized. Based on laboratory observations of adult female nematode behaviour in thin water films on agar plates, the likely pattern of preparasite dispersal can be deduced. The adult female, full of active preparasites, moves a few mm, stops, and moves backwards a mm or so. This flexes the barrel-shaped vagina, located approximately in the middle of the body. With this flexing, a few preparasites are expelled from the vagina at a time. The female then moves another cm or two and repeats the process. This probably disperses the preparasites in sinuous rows in the *Culicoides* larval habitat, improving the likelihood that some will encounter hosts.

The distribution of numbers of *H. vivipara* in individual *Culicoides* larvae (apparently *C. puncticollis*) dissected within natural populations sampled at different points in time varied from regular (variance<mean) to contagious (variance>mean) [Lebedeva, 1988; Lebedeva & Gafurov, 1994]. A regular distribution of parasites per host larva was observed at a low parasitism level (about 6%), while a contagious distribution was observed at high parasitism levels (up to 50%). A random distribution (variance approximates mean) was observed in some populations with low or moderate parasitism levels (1–17%). The authors felt that the random distribution corresponded to the initial period of infestation or to the period of primary emergence of mermithids from hosts [Lebedeva, 1988].

Carryover into the adult host, dispersal and host range

Adult *C. variipennis* and *C. sonorensis* sometimes have been collected harbouring *H. magnapapula* [e.g. Mullens & Schmidtmann, 1982]. Parasitized adult midges in fact may be more common than is appreciated, since they are best detected by dissection, especially in species with a dark abdominal cuticle. Paine & Mullens [1994] collected large numbers of adult *C. sonorensis* from (emergence traps) and near (light traps, CO_2-baited suction traps) habitats that had large numbers of both the nematode and the host. Only 3 of 5,308 adults were found to harbour adult nematodes on dissection (0.06% adult parasitism). It therefore appears that carryover into the adult stage was rather rare for that system. In the laboratory, however, Mullens & Velten [1994b] normally observed carryover into adults (exposed as late second or early third instars) at rates of 0.5–2.5%, and high rates of nematode carryover into the adult midge (up to 17%) were created by exposing older (4th instar) larvae to the preparasites.

Compared to this, there are some contrasting examples of carryover of *H. vivipara* into adult midges. Using dissections, Bekturganov [1991] in Kazakhstan collected over 11,000 specimens of *H. vivipara* from larvae of five *Culicoides* species, but never observed carryover into adults and concluded that all the mermithids emerged as adults from the larval hosts. In contrast, in some *Culicoides* populations in Uzbekistan the percentage of adults infested with *H. vivipara* was moderate to very high: 11% [Lebedeva, 1988; Lebedeva & Gafurov, 1994] and 48% [Saidalieva, 1985]. However, all authors who studied *Heleidomermis* have agreed that most of parasites emerge from the host larvae, killing those hosts in the process.

The nematode frequently is found in habitats that last only a few weeks or months, and dispersal among disjunct habitats via adult midges would be a logical mechanism. If adult *C. sonorensis* parasitism by *H. magnapapula* is uncommon, that would help explain its very patchy distribution in habitats that harbour the host midge [Paine & Mullens, 1994]. Rubzov [1974b] observed similar patchy distributions of various mermithids that parasitize aquatic Diptera, including biting midges, and explained them the same way. The other means of mermithid dispersal could include washing larval hosts from one habitat to another, or movement of nematodes in mud adhering to the feet of water birds. Adult *Heleidomermis* spp. do not lay eggs, which are a relatively persistent life stage for some other mermithids such as *Romanomermis* [Platzer et al., 2005]. Finally, *H. magnapapula* might not be host-specific, and could conceivably parasitize other *Culicoides* species.

This certainly appears to be true for *H. vivipara*, which has been reported from 13 naturally-collected host *Culicoides* species in European Russia, Buryatia, Kazakhstan and Uzbekistan (see Tables 1 and 2; the records from Buryatia were originally attributed to *H. ovipara*). Host species of *Culicoides* include members of four

subgenera, *Beltranmyia*, *Monoculicoides*, *Culicoides* s. str. and *Oecacta*. In addition to this, *H. vivipara* was recorded from unidentified Ceratopogonidae other than *Culicoides* [Lebedeva, 1988; Lebedeva & Gafurov, 1994].

Mullens et al. [1997] obtained eggs from gravid, field-collected (mated) adult females of a number of other *Culicoides* spp. in California and attempted to rear them on agar using a diet of either bacterial-feeding nematodes or microbe-rich water from *C. sonorensis* rearing pans [Kettle et al., 1975; Mullens & Velten, 1994a]. *Heleidomermis magnapapula* preparasites very readily entered the larvae of any *Culicoides* spp. they encountered. The *Culicoides* species could be reared to varying degrees on the agar. Of nine *Culicoides* spp. reared on agar and exposed to preparasites, the nematodes emerged successfully from three species and showed some development in a fourth species. Interestingly, as in *H. vivipara*, the species that allowed nematode development were in several subgenera: *C. sonorensis* and *C. occidentalis* (*Monoculicoides*), *C. cacticola* Wirth & Hubert, 1960 (*Drymodesmyia*), and *C. lahontan* Wirth & Blanton, 1969 (*Culicoides* s. str.). Larvae of other Diptera commonly found in polluted mud habitats along with *C. sonorensis*, such as *Psychoda* (Psychodidae) or *Eristalis* (Syrphidae), were ignored by the preparasites, even if they made direct contact with them in dishes of water. Preparasites did penetrate larvae of *Chironomus*, but were rapidly encapsulated. Similarly, *H. vivipara* also is restricted at least to Ceratopogonidae. This species was never found in other invertebrates in sites where it was abundant; preparasites ignored the larvae of Culicidae in a laboratory experiment [Bekturganov, 1991].

Seasonality

Like most of their midge hosts, *H. magnapapula* and *H. vivipara* can complete multiple generations per year. With *H. magnapapula*, experimental evidence in the laboratory has shown that the mermithid development within the host (and embryogenesis) varies with temperature. Development in the host requires from 9.1 days at 32.2°C to 35 days at 15.6°C, for a total 214 degree days above a lower developmental threshold temperature of 8.9°C, and development proceeds at the same relative pace as its host larvae [Mullens et al., 1995]. When second instar host larvae are exposed, emergence of the nematodes occurs about 10–14 days later at temperatures of about 23°C [Mullens & Velten, 1994b]. Assuming only slight delays in mating or locating hosts and adding 3–4 days for embryogenesis, the entire life cycle of *H. magnapapula* at 23°C thus requires about 16–19 days. As with its host, it is very likely that *H. magnapapula* reproduces continuously in *C. sonorensis* in the mild southern California climate, while it overwinters within the host larvae in colder areas; overwintering, parasitized *C. variipennis* larvae were collected in early April in New York State [Mullens & Rutz, 1982]. In general, the highest levels of field parasitism by *H. magnapapula* occur in late summer and fall, when host densities are also highest [Mullens & Rutz, 1982; Paine & Mullens, 1994].

While temperature influences were not specifically discussed, according to Bekturganov [1991], the life cycle of *H. vivipara* in SE Kazakhstan required 20–25 days (presumably at summer temperatures). Despite this, and probably considering host voltinism patterns, he surmised the nematode completed two main generations per year, in April and September in desert and semidesert regions, and in June and September in the more mesic foothill environments. The parasitic stage lasted 15–20 days, and the life cycle of the parasite was synchronized with appearance of large numbers of *Culicoides* larvae. According to Lebedeva [1993], the parasitic stage of *H. vivipara* lasted 15–40 days; overwintering occurred in the host (mud temperatures decreased to 1.5°C), and in this case the parasitic stage lasted up to 4 months.

In some habitats of Kazakhstan, high parasitism levels (18–30%) were observed both in spring and in autumn [Bekturganov, 1991]. Lebedeva described seasonal fluctuation in parasitism levels, which differed between the plain and mountain zones of Uzbekistan [Lebedeva, 1988; Lebedeva & Gafurov, 1994]. On the plains, *H. vivipara* was not found in biting midges during summer; the percentage of parasitized hosts increased from September (4–14%) through March (up to 50%), but decreased after April. In contrast, in the foothills and in the mountains parasitized host larvae were not detected during winter, but were found during summer. The maximum parasitism level was observed in spring (36%, mountains), or in autumn (50%, foothills).

Presumably, the periods without observed *H. vivipara* parasitism may reflect limitations imposed by abiotic conditions, such as high water temperature (up to 33°C in some habitats), a summer host diapause, or freezing and drying of many mountain habitats in winter [Lebedeva, 1988]. A persistent life stage is apparently absent in *Heleidomermis* spp. Freezing, drying or high temperatures may reduce preparasite infectivity, survival and development of (semi)aquatic eggs and larvae, described in many mermithid taxa [Polozhentsev et al., 1977; Rubzov, 1977; Petersen, 1985; Artyukhovsky, 1990; Kharchenko, 1999].

Mermithids and biological control of *Culicoides*

Biological control, as modified from Van Dreische & Bellows [1996], is defined here as the manipulation of natural enemies (parasites, pathogens, predators, antagonists or competitors) with the goal of suppressing pest species (and reducing damage). Judging from the life history traits and host-parasite relationships, mermithids are parasitoids [Viktorov, 1976; Begon et al., 1986; Van Driesche & Bellows, 1996; Schowalter, 2006], and the large body of theoretical and practical principles developed on insect parasitoids (e.g. parasitic hymenopterans, tachinids) also could be useful in developing mermithids as biological control agents. Manipulation thus might include direct inoculative or inundative releases of a natural enemy into a habitat,

alteration of an existing habitat to favour the activity of the natural enemy, etc. This obvious possibility has been mentioned many times for *H. vivipara* since the discovery of mermithids in *Culicoides* [e.g. Glukhova, 1967; Mirzaeva, 1971; Wirth, 1977a; Gafurov & Saidalieva, 1984; Lebedeva, 1988; Bekturganov, 1991].

Culicoides spp. are quite diverse in their ecology, but in general do share some features [Kettle, 1977]. They are mostly either aquatic or semi-aquatic as immatures, where they feed on detritus, microorganisms, or other small invertebrates such as bacterial-feeding nematodes. So far, the *Culicoides* species that harbour *Heleidomermis* often live in sites that are both spatially separated from other similar habitats and are semi-permanent or temporary in nature. An animal watering trough overflow, for example, might last only a few weeks in late summer, but rather quickly could be colonized by both *Culicoides* and *Heleidomermis*. The *Culicoides* that use such sites are excellent dispersers and colonizers, taking advantage of habitats that might disappear at any time. A mermithid that could utilize such hosts would be under selective pressure to mature with equal speed.

In general, then, this helps explain the very unusual *Heleidomermis* life cycles. The mermithids mature in their hosts in as little as 10 days and emerge from the host already adults, ready to mate. A few days later, females give birth to preparasites, ready to invade a host and produce another generation approximately as fast as the host species can. *Heleidomermis* spp. thus avoid delays experienced by most mermithids, such as emerging from the host as a juvenile, moulting to the adult, and laying eggs that may take weeks or months to hatch.

Species of *Heleidomermis* do have some significant and inherent limitations for biological control of *Culicoides*, as listed below.

1) <u>Lack of a persistent life stage</u>. There is no persistent or relatively environmentally-resistant egg stage as occurs in some mermithids, which might allow *Heleidomermis* to persist in a dry habitat or to be stored by people pending distribution in a natural habitat.

2) <u>Lack of an *in vitro* rearing system</u>. There is currently no way to rear *Heleidomermis* outside of a living host. In fact, very few *Culicoides* spp. have been colonized that might be used for *in vivo* rearing. *In vitro* rearing systems, even if developed, would likely be quite involved and difficult, as has proven true with attempts to rear *in vitro* the mosquito mermithid *Romanomermis culicivorax* Ross & Smith, 1976 [e.g. Castillo et al., 1982].

3) <u>Mechanism of sex ratio determination</u>. The mechanism of sex ratio determination probably evolved to prevent overexploitation of the host resources through time. As discussed above, a high level of exposure of *Culicoides* larvae to preparasites results in superparasitism, direct host kill and/or overproduction of males, all of which would reduce the numbers of mermithids available to control the next generation of *Culicoides*. It therefore would be unreasonable to expect *Heleidomermis*, or in fact most mermithids, to parasitize a very high proportion of available hosts continuously through time.

4) <u>Limited capability for dispersal</u>. As discussed above, most *Heleidomermis* individuals typically emerge from host larvae, and thus remain in the same habitat. This helps explain the patchy natural distribution and can limit their ability to invade new habitats and suppress *Culicoides* numbers there.

5) <u>Density-dependent host regulation</u>. Available evidence suggests that species of *Heleidomermis* reach relatively high parasitism levels only in dense host populations. They evidently are limited in host location at low population densities. If true, *Heleidomermis* would be predicted to be most effective in controlling those *Culicoides* species whose larvae reach high abundance in local specific habitats (such as many representatives of *Beltranmyia* and *Monoculicoides*).

The above limitations except the first one are widely known for the mermithids as a whole [e.g. Rubzov, 1974a, 1977, 1978; Gafurov, 1997; Kharchenko, 1999].

Despite that, opportunities for biological control do exist, and more research is justified to investigate both natural *Culicoides* mortality caused by *Heleidomermis* and its possible manipulation to our advantage. *Heleidomermis* spp. have several advantages as biological control agents as enumerated below.

1) <u>Fast development rate</u>. As mentioned above, they can mature fast enough to utilize the fastest-developing *Culicoides* spp., even in temporary habitats.

2) <u>Host specificity</u>. *Heleidomermis* is moderately host-specific in comparison with other mermithids [Gafurov, 1997]. While the host-specificity simultaneously limits their application to certain target *Culicoides* spp., non-target impacts are probably of little concern.

3) <u>Ability to use alternate *Culicoides*</u>. Despite moderate host specificity, *Heleidomermis* are not strictly limited to a single host species. Indeed, Rubzov [1974a] stated that limitation to a single host is not common for aquatic mermithids. Available evidence suggests they will attack and/or use a number of *Culicoides* spp., given the opportunity. Within a suitable habitat, therefore, the ability to utilize multiple hosts in the genus might be an advantage in persistence.

4) <u>Lethality</u>. While lethality is not absolutely necessary for a biological control agent, the nematodes will inevitably kill hosts that permit their maturation. For *Heleidomermis* spp., both parasitism and death often occur in the larvae of *Culicoides*, preventing them from becoming adults to perhaps feed on vertebrates or transmit disease agents.

5) <u>Parasitic castration and dispersal adaptations</u>. Even if the mermithids carry over into the adult stage, parasitized adults probably are parasitically castrated. There is little research on whether parasitized adults of *Culicoides* might blood-feed. Glukhova [1967] did note mermithid parasitism in an adult *Leptoconops* that was blood-engorged, and Jeu [1977] dissected 152 parasitized, blood-engorged *C. riethi*. In that case, parasitism clearly did not prevent blood-feeding, as may also be true for at least some other blood-feeding Diptera [Nickle, 1972]. However, it also is likely that behaviour of adult *Culicoides* might be altered by parasitism, for

example causing males and/or females to behave as though they were gravid females and seek out a developmental site rather than a vertebrate host. Alterations in host behaviour, often benefitting the parasite, are known in other insect-mermithid interactions [e.g. Wülker, 1970; Rubzov, 1974a; Vance, 1996].

6) Habitat and host range probably are greater than we now understand. The majority of *Heleidomermis* reports have been from saturated mud in somewhat polluted habitats with free-standing water. However, there are some intriguing suggestions that species exist that utilize different types of habitats. Glukhova [1967] reported that 18–27% of *C. grisescens* larvae from paths in a *Sphagnum* and pine forest in Karelia were parasitized by Mermithidae. Mirzaeva [1971] reported high mermithid parasitism (up to 66.6% larvae infested) in *C. helveticus* collected from a large permanent marsh near a stream in a forest in Buryatia. Larvae of *C. obsoletus* and closely related species in both the Nearctic and Palaearctic may be found in moist (but not extremely wet) terrestrial environments such as damp leaf litter [e.g. Battle & Turner, 1971], and adults of *C. obsoletus* were quite commonly parasitized by mermithids in England [Boorman & Goddard, 1970].

7) Season-long activity. Mermithid parasitism can occur season-long within a habitat, as discussed above. Poinar & Sarto i Monteys [2008] reported *H. catalonien-sis* parasitism in northeastern Spain at least from August through October. Thus far it is quite unusual for other natural enemy groups from *Culicoides* to cause either similarly high levels of *Culicoides* mortality or cause it over a significant period of time (with the possible exception of some pathogens), although more work on that definitely needs to be done [Wirth, 1977a, 1977b].

8) Mass production potential. It may well be possible to rear and release useful numbers of *Heleidomermis* reared *in vivo*, using the *Culicoides* species we can rear in the laboratory as hosts.

9) Substantial host impact. Mermithids can cause very substantial host mortality in *Culicoides*. Conservative estimates based mostly on parasite emergence show parasitism frequently ≥ 20–40%. Some high prevalence reports may reflect retarded development of parasitized larvae and artificially high point-prevalence. Retarded development was observed in *Culicoides* larvae parasitized by *H. vivipara* [Saidalieva, 1985; Bekturganov, 1991] and probably also in *C. sonorensis* parasitized by *H. magnapapula* in both field and laboratory [Paine & Mullens, 1994; Mullens & Velten, 1994b]. However, estimates of host mortality based on successful mermithid emergence from field-collected hosts (populations sampled repeatedly through time) are quite conservative overall, and underestimate true impact by up to 50% [Mullens & Luhring, 1998]. Accurate estimates of host impact require collecting hosts for multiple weeks, and appreciating that hosts may be killed outright by the parasites, even if they do not survive to allow the parasite to emerge [e.g. Mirzaeva, 1971; Mullens, unpublished]. In some systems, we probably are missing a large part of the host-parasite relationship story if we fail to consider carry-over into adults.

Preliminary field release trial

An experimental release trial was done by one of us (B.A. Mullens) in a 3 m wide by 5 m long by 0.5 m deep pond in southern California, using laboratory-reared *H. magnapapula* and eggs of *C. sonorensis*. This was done to test the concept of inoculative or inundative release, as has been frequently utilized with entomopathogenic nematodes such as different species of *Steinernema* Travassos, 1927 or *Heterorhabditis* Poinar, 1976 [Grewal et al., 2005]. The pond received 10 liters of cow manure slurry weekly to stimulate microbial activity that would serve as food for larval *C. sonorensis*. Eight plastic field enclosures were placed crossing the waterline of the pond. The 40 cm by 55 cm enclosures had fine mesh nylon screen sides (0.3 mm openings) to admit light but exclude passage of adult *Culicoides* spp. (Fig. 5). Enclosure edges extended 10 cm into the substrate to prevent movement of the surface-dwelling larvae [Barnard & Jones, 1980]. Enclosure tops were modified into emergence chambers. Emerging *Culicoides* drowned in soapy water saturated with NaCl, where they were collected and identified to species (*Culicoides*) or genus (other Ceratopogonidae). They were counted on day 15, every 2–3 days from day 24–57, and weekly from day 57–104. Emerging adult *C. sonorensis* were dissected to check for internal mermithids.

Approximately 1,500 *C. sonorensis* eggs were added to edge mud in each enclosure by removing the emergence heads on day 0 (October 20) and another 1,500 eggs were added on day 7. On day 7, a mixture of 40 laboratory and field-collected, adult female, mated *H. magnapapula* were added similarly to each of 4 enclosures, while the remaining 4 enclosures received no mermithids. Treated enclosures thus received approximately 40,000 preparasites each [Mullens & Velten, 1994b]. On day 21, surface mud from just below waterline in one release (treated) enclosure and one negative control enclosure was harvested (destructively sampled), and 4th instar *C. sonorensis* larvae were extracted by sieving. These were placed individually into the wells of three ELISA plates (288 larvae for each of the negative control and treated enclosures) for mermithid emergence.

For the destructively-sampled enclosures from day 21, 29% of the host larvae from the treatment enclosure yielded emerged *Heleidomermis*, with a sex ratio of 282 males and 11 females (26 : 1). Up to 12 very small male *H. magnapapula* emerged from a single host larva, a level of superparasitism not normally seen in the field [Paine & Mullens, 1994]. No mermithids emerged from the negative control larvae. The heavily male-biased sex ratio, and high numbers emerging from some individual hosts, indicated a very high level of host exposure in the treated enclosures. Paine & Mullens [1994] showed an overall sex ratio of 2.4 males per female from field-collected hosts.

Numbers of emerging adult *C. sonorensis* were significantly higher in the untreated enclosures (Fig. 6)



Fig. 5. Enclosure traps used in experimental release trial of *Heleidomermis magnapapula* against *Culicoides sonorensis* in southern California. Photo by B. Mullens.

Рис. 5. Полевые изоляторы, использованные в опытах по заражению мокреца *Culicoides sonorensis* мермитидой *Heleidomermis magnapapula* в южной Калифорнии. Фото Б. Мюлленса.

(ANOVA, $p<0.01$). Overall, a mean ($\pm$ standard deviation) of 167.7 ± 35.9 *C. sonorensis* emerged from the control enclosures, versus 27.0 ± 4.3 from the treated enclosures, a reduction of 84% due to the mermithid release. None of the emerging adult *C. sonorensis* was parasitized by mermithids upon dissection.

A total of eight *C. crepuscularis* emerged in the first 43 days of the trial, with no further emergence afterwards. Seven came from the treated enclosures and one from the control enclosures, which were not compared statistically. Numbers of emerging adult *Dasyhelea* Kieffer, 1911, a non-blood-feeding ceratopogonid, were high enough over the first 41 days to analyze statistically, and did not differ between the treated and control enclosures ($p>0.1$). Emergence of *C. crepuscularis* and *Dasyhelea* adults early in the trial reflected larvae that were in the mud when the enclosures were placed, while no *C. sonorensis* adults emerged before day 15. The rather extended adult *C. sonorensis* emergence period, and the relatively low numbers emerging even from the untreated enclosures probably reflected moderately cool fall conditions, but also suboptimal *Culicoides* developmental conditions within the enclosures. Still, the differences between treated and control emergence were very obvious.

This preliminary trial showed that an inundative release could drastically reduce subsequent short-term *Culicoides* emergence from small habitats, but some midges escaped parasitism even under the very high exposure conditions. The lack of carryover into emerging adult *C. sonorensis* agreed with Paine & Mullens [1994]. However, the introduction of the mermithids also was intentionally timed to coincide with presence of early stage larvae, rather than later stages that might have more tendency to carry mermithids through to the adult stage. With this high exposure level, parasitism based on emergence from lab-held hosts (29%) very drastically underestimated actual impact of the releases (84%) and was extremely conservative. This was probably due to direct, premature host death caused by invasion of host larvae by large numbers of preparasites.

Future biological control possibilities for *Heleidomermis*

Without efficient and cost-effective ways of producing large numbers of mermithids, inundative releases (i.e. their use as a "biological insecticide") are probably not feasible. A site of 10–20 metres in diameter, such as a fairly small dairy wastewater pond, might require thousands of mermithids to severely reduce *Culicoides* numbers. It is, however, quite possible to rear reasonable

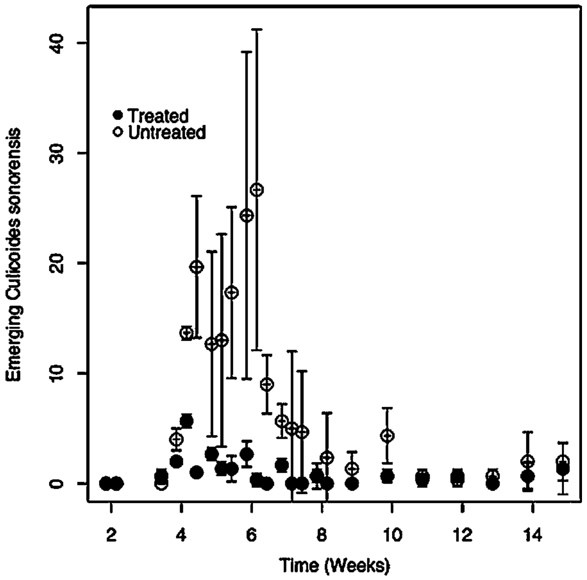


Fig. 6. Numbers (mean ± standard deviation) of adult *Culicoides sonorensis* emerging from field enclosures supplied with eggs of *C. sonorensis* on day 0 and day 7. Enclosures each received 40 females of *Heleidomermis magnapapula* on day 7 (treated) or nothing (untreated).

Рис. 6. Численность (среднее значение ± стандартное отклонение) взрослых особей *Culicoides sonorensis*, появляющихся в полевых изоляторах, куда добавлялись яйца *C. sonorensis* в день начала эксперимента и через семь дней после этого. В каждый изолятор через 7 дней после начала опыта было помещено 40 самок *Heleidomermis magnapapula* (вариант “treated”; в варианте “untreated” мермитиды не добавлялись).

numbers (hundreds of females per week) of *H. magnapapula* using the laboratory host *C. sonorensis* reared at a modest scale. It currently is not known whether a similar approach might be used for other species of *Heleidomermis*, but this deserves investigation.

Gubaidulin et al. [1987] and Bekturganov [1991] also recommended using the material from known habitats with high parasitism level to infest other *Culicoides* sites. Field-collected mermithids, *Culicoides* larvae and shore substrate from infested bodies of water were considered as possible material for introduction. However, it is not clear if this technique could be effective using *Heleidomermis*, and no experimental data were presented by the above-cited authors.

Inoculative releases of perhaps only a few mermithids, or parasitized hosts, into selected sites could be helpful for *Culicoides* suppression. In sites where both host and parasite are established, *H. magnapapula* naturally kills about 30–50% of the *C. sonorensis* larvae in a typical dairy wastewater pond in southern California through the peak summer-fall period. This level of mortality could easily be considered an important part of an integrated pest management program.

Mermithids are key natural enemies of many *Culicoides* species and regularly parasitize an appreciable proportion of different species in certain types of habitats. These mermithids have evolved fascinating and complex strategies for host utilization and persistence, and are worthy subjects for pure parasitologists. The scientific community has only barely begun to investigate these important relationships, and more work doubtless will uncover new species and interesting relationships between mermithids and biting midges that are widespread, diverse, and perhaps important to understand in order to enhance pest suppression.

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